III. BIOLOGIC EFFECTS OF EXPOSURE

Extent of Exposure

Nitriles are organic compounds that contain a cyano group, C≡N, as the characteristic functional group. Under selective hydrolysis conditions, nitriles may be hydrolyzed to the corresponding acid amides, whereas complete hydrolysis yields carboxcylic acids. The selective reduction of nitriles may yield imines, which can be hydrolyzed to aldehydes, and the complete reduction yields primary amines. In addition, selected nitriles, particularly cyanohydrins, will readily dissociate to form cyanide and the corresponding side chain derivative. Most nitriles are expressed by the general formula RC≡N in which R may be any saturated or unsaturated univalent organic radical. Nitriles in which an alpha-hydroxy group is bound to a carbon atom of the side chain are referred to as cyanohydrins [1].

Selected mononitriles, cyanohydrins, and dinitriles are included in this document. Their selection was based on the extent of production and use in industry and the degree of toxicologic hazard. The synonyms for selected nitriles are listed in Table XII-1.

The mononitriles selected for inclusion are acetonitrile, propionitrile, n-butyronitrile, and isobutyronitrile. These are saturated aliphatic nitriles with molecular weights ranging from 41.1 for acetonitrile to 69.1 for the butyronitriles. All are colorless liquids having varying solubilities in water. Vapor pressures for these nitriles at 20 C range from 14 mmHg for n-butyronitrile to 73 mmHg for acetonitrile [1].

The two cyanohydrins included are glycolonitrile and acetone cyanohydrin. Glycolonitrile, the alpha-hydroxy derivative of acetonitrile, has a molecular weight of 57.1; acetone cyanohydrin, the alpha-hydroxy derivative of isobutyronitrile, has a molecular weight of 85.1. Both glycolonitrile and acetone cyanohydrin are water-soluble colorless liquids. Acetone cyanohydrin has a vapor pressure of 0.8 mmHg at 20 C [2].

The dinitriles included are malononitrile, succinonitrile, adiponitrile, and tetramethylsuccinonitrile. The molecular weights for these dinitriles range from 66.1 for malononitrile to 136.2 for tetramethylsuccinonitrile. Three of these dinitriles are odorless solids; adiponitrile is a colorless and odorless oily liquid. Adiponitrile, malononitrile, and succinonitrile range from slightly soluble to soluble in water. Tetramethylsuccinonitrile is soluble in alcohol.

When heated to decomposition, nitriles emit toxic fumes containing cyanides [3]. Other physical and chemical properties of the mononitriles, cyanohydrins, and dinitriles are included in Table XII-2.

Nitriles can be synthesized by various processes; however, the following six processes provide examples of the most common methods used in industry.

(1) Dehydration of acid amides prepared by heating an equimolar mixture of a carboxylic acid and ammonia in the presence of a suitable catalyst:

$$(RCOOH + NH_3 \text{ ------> } RC{\equiv}N{+}2H_2O)$$

Acetonitrile can be prepared by reacting acetic acid and ammonia at 400-500 C in the presence of a dehydration catalyst consisting of 20% phosphoric acid and aluminum oxide [4]. Adiponitrile can be prepared by heating adipamide with acetic anhydride in the presence of cobalt [4].

(2) The dehydration of an ammonium salt of an organic acid with

$$(RCO_2NH_4 + P_2O_5 \text{ ----> } RCONH_2 + 2HPO_3)$$

heat plus a catalyst to produce an acid amide that is then dehydrated:

$$(RCONH_2 + P_2O_5 \text{ ----> } RC{\equiv}N + 2HPO_3)$$

Acetonitrile may also be prepared by heating ammonium sulfate or diammonium monohydrogen phosphate with acetic acid at 200 C.

(3) The reaction of alkylhalides with sodium or potassium cyanide in an aqueous-alcoholic solution:

$$(RX + NaCN \text{ ----> } RC{\equiv}N + NaX)$$

Propionitrile may be prepared by the reaction of ethyl chloride with potassium cyanide. Adiponitrile may be synthesized by reacting 1,4-dichlorobutane with sodium cyanide.

(4) Adiponitrile may be prepared by reacting butadiene with hydrogen cyanide:

$$CH_2 = CHCH = CH_2 + HCN \text{ ------> } (CH_2)4(CN)2$$

or by the electrodimerization of acrylonitrile:

$$2CH_2 = CHCN \xrightarrow{H_2} (CH_2)4(CN)2$$

(5) Malononitrile is prepared by continuous introduction of preheated acetonitrile and cyanogen chloride into a tube reactor until the reaction mixture reaches a temperature of approximately 780 C:

$$CH_3CN + ClCN \text{ ------> } CH_2(CN)2 + HCl$$

Published production estimates are available for acetonitrile, acetone cyanohydrin, and adiponitrile [5]. In 1962, 2.1 million pounds of acetonitrile were produced [6]. In 1964, 3.5 million pounds were consumed. In 1975, 573 million pounds of acetone cyanohydrin were produced, and its production is expected to increase at an average rate of 9% through 1980 [5]. In 1976, at least 150 million pounds of adiponitrile were produced [5]. Imports of malononitrile, which currently is not manufactured in the United States, are estimated at up to 60,000 pounds annually (I Gottleib, personal communication, February 1978).

Nitriles are adaptable to industrial applications because of their versatile chemical reactivity. The nitrile functional group can be converted to a primary amine; a carboxylic acid; or the corresponding amide, aldehyde, alcohol, or ester derivatives. Dinitriles can be converted to compounds with difunctional groups for use in a variety of processes and products, including plastics, dyestuffs, synthetic intermediates, and pharmaceuticals [7-9]. Industrial uses are listed in Table XII-3.

Industrial use for nitriles in the manufacture of plastics, synthetic fibers, elastomers, and solvents was stimulated by the growth of the petrochemical industry after World War II [10]. Acetonitrile was introduced to the commercial market in 1952 [11], but high-technology commercial development was inhibited for several years by economic factors. In 1967, new designs in the electrolytic synthesis of fine chemicals improved the efficiency of nitrile production, notably adiponitrile, and effectively increased the marketability of nitriles for industrial uses [12].

Acetonitrile is used as a solvent in extractive distillation that separates olefins from diolefins, butadiene from butylene, and isoprene from isopentane. Propionitrile is used as a solvent for the separation of hydrocarbons and in refining petroleum fractions. Isobutyronitrile is used as a catalyst in the polymerization of ethylene. Isobutyronitrile has been tested in the petroleum industry as a gasoline additive [13].

Glycolonitrile is an intermediate in the synthesis of bactericides and fungicides. Acetone cyanohydrin is used primarily in the preparation of methyl methacrylate, which is polymerized to form various plastics, including Plexiglas [4].

Adiponitrile is used as a raw material in the manufacture of synthetic fibers. The cyano groups of adiponitrile are hydrogenated to form hexamethylenediamine, a compound used in the production of nylon [14].

Malononitrile is used primarily as a chemical intermediate in the manufacture of thiamine and in the manufacture of pteridine-type anticancer agents; it also has application in the manufacture of photosensitizers, acrylic fibers, and dyestuffs and as an oil-soluble polar additive in lubricating oil [8,9].

The major occupational exposures to nitriles occur principally by inhalation of vapor or aerosols and by skin absorption. The likelihood of such exposures increases during the handling, transferring, and quality control sampling of nitriles. In addition, an increased risk of exposure is possible during maintenance operations and repair of equipment; on and after entry into tanks, vessels, or other confined spaces; or when emergency or nonroutine procedures are required [4].

Using data from a 1972-74 National Occupational Hazards Survey, NIOSH estimates that approximately 26,000 employees are potentially exposed to the selected nitriles in the occupational environment. It is estimated that acetonitrile exposures account for 24,000 of this figure. Occupations with the greatest potential for exposure to these selected nitriles are listed in Table XII-4.

Historic Reports

Several nitriles were discovered in the 19th century. Propionitrile was first prepared by Pelouze [13] in 1834. Malononitrile was synthesized by Henry in 1886 [15], and adiponitrile reportedly was identified by Henry in 1901 [16]. Naturally occurring nitriles were isolated from several plants in 1874 [17].

Giacosa [18], in 1883, mentioned that Pelikan and Maximowitsch had already investigated the toxic effects and possible therapeutic uses of nitriles. Giacosa was more interested in studying the transformation of such compounds by mammalian systems. He administered propionitrile subcutaneously (sc) to animals (species not mentioned). He observed the odors, colors, and precipitates of metabolites in exhaled air, urine, and feces. Some propionitrile was exhaled unchanged; some was metabolized and excreted in the urine as propionic acid. Giacosa gave a dog acetonitrile for a few days. It was first given in capsules with the food at doses up to 65 mg/kg/day and later by sc injection at doses up to 100 mg/kg/day. Other than the dog's refusal to eat the dosed food, no adverse effects were noted. From his own studies and reviews of those of other investigators, Giacosa concluded that the elimination of acetonitrile and its metabolic products was through the exhaled air and the urine.

Giacosa's discovery [18] of sulfocyanic acid in the urine of animals poisoned with acetonitrile or hydrogen cyanide led to the use of sulfur compounds as antidotes for hydrogen cyanide poisoning. In 1907, Hunt [19] wrote that Giacosa's discovery of sulfocyanic acid in the urine after administration of acetonitrile (and some other nitriles) was the beginning of pharmacologic knowledge of acetonitrile.

Meurice [20], in 1900, reported on the toxic effects of various nitriles in pigeons. Acetonitrile injected intramuscularly (im) caused no reaction at a dose of 500 mg/kg body weight, incomplete paralysis at 1,000 mg/kg, marked paralysis at 2,000-3,500 mg/kg, and death within 23 hours at 4,000 mg/kg. Propionitrile at 1,250 mg/kg caused death in 1.5 hours. At 1,100 mg/kg, n-butyronitrile caused convulsions and death in 1 hour. Isobutyronitrile at 2,500 mg/kg caused paralysis and death in 1.5 hours.

In 1906, Brissemoret [21] discussed some pharmacologic properties of several nitriles including acetonitrile and propionitrile. They were characterized as causing labored breathing, convulsions, asphyxia, and gastrointestinal irritation.

Hunt [22], in 1905-1906, while studying contemporary theories on thyroid function, injected acetonitrile sc into white mice that received powdered thyroid in the diet. Results indicated that the powdered thyroid modified metabolism so that the toxic effects of acetonitrile were not as severe as those seen in mice that were provided a diet without thyroid supplementation. This finding was the basis for what became known as the acetonitrile test for thyroid function in which the blood of patients with hyperthyroidism increases the resistance of mice to poisoning by acetonitrile [23].

In 1932, Marine et al [24] described experimental studies of nitriles that led to an initial understanding of the "essential" cause of thyroid hyperplasia. Further studies on acetonitrile by Marine and Rosen [25], in 1934, were significant in stimulating future inquiry into the cause of hyperthyroidism.

Hyden and Hartelius [26], in 1948, reported that malononitrile injected intravenously (iv) stimulated the formation of nucleoproteins in pyramidal cells of the cerebral cortex in rabbits. They also compared pyramidal cells of 11 "schizophrenic" persons with those of 4 "normal" persons, and they found that the pyramidal cells of schizophrenic persons were poorly developed compared with those of the other persons. Hyden and Hartelius also discussed previously unpublished clinical studies of Hyden and Reuterskiold on the use of malononitrile in the treatment of mental illness. Their work was followed by the clinical studies of MacKinnon et al [27] in 1949, Meyers et al [28] in 1950, and Hartelius in 1950 [29].

In 1964, Marigo and Pappalardo [30] described in detail the death of a patient who had received daily im injections of succinonitrile (200 mg) for

about 3 weeks as part of a therapeutic regimen for treatment of polyarthritis and bronchial inflammation. The 53-year-old man was hospitalized after he developed vomiting, psychomotor agitation, mental disorientation, and cold sweating; he died 2 hours later during one of many tonic-clonic convulsions.

A post-mortem examination revealed few abnormalities [30]. Unusual gross findings included cerebral and pulmonary edema and congestion in the brain, lungs, and gastrointestinal tract. Microscopic examination yielded evidence of fatty and vacuolar degeneration of the liver and marked tubulonephrosis. Recognition of the odor of bitter almonds when the brain was removed from the skull during the autopsy led to the initiation of a forensic toxicologic investigation of the man's death. The delayed onset of death made the possibility of primary poisoning from cyanide unlikely but suggested poisoning by a secondary source of cyanide, eg, a nitrile. The discovery that the patient had been undergoing therapy with succinonitrile for polyarthritis and bronchial inflammation, together with the presence of cyanide in the urine and various viscera, strengthened the conclusion that succinonitrile probably was the lethal agent. Marigo and Pappalardo proposed that detoxication of cyanide by the enzyme rhodanase in the patient failed to keep pace with the accumulation of cyanide.

This report [30] implicated succinonitrile as the responsible agent in a fatal poisoning. Using this information, the authors retrospectively analyzed three other observed fatal cases of sudden death from supposed toxic, but unspecified, agents. Because succinonitrile was involved in all three cases, two in therapeutic doses for mental depression (one following 6 days of 350 mg im injections and the other following 15 days of 200 mg/day im injections) and one as an accidental injection of 500-600 mg, the compound was considered to have contributed to the deaths. A fourth case, not personally observed by the authors but reported by them, was of an accidental ingestion of 400-500 mg of succinonitrile that led to death shortly thereafter. The symptoms were similar in all cases, with convulsions the common antecedent to death.

Effects on Humans

The effects of nitriles on humans (summarized in Table III-2) are described for compounds within three classifications: (a) mononitriles, (b) cyanohydrins, and (c) dinitriles. For each compound, workplace exposures or clinical cases are described first, followed by human experimental studies, when available.

(a) Mononitriles

In 1955, Grabois [31] described an incident in which 16 workers in a chemical manufacturing plant were accidentally exposed to ACETONITRILE

vapor while brush painting the inside walls of a storage tank with corrosion-resistant resinous primer paint. The paint consisted of an epoxy resin, a polysulfide resin, a polymerization catalyst, and an air inert filler. Acetonitrile was used as a thinner for the paint and was the only volatile solvent in the mixture. The paint was brushed on the inside of the tank by three workers at a rate of 2 gallons/hour.

On the 2nd day of the operation, about 7 gallons of paint were used. The paint was warmed to at least 25 C to facilitate application. In addition, the supply of fresh air to the compartments was reported to have been interrupted for about 15 minutes toward the end of the day. Fifteen workers, including 11 not involved in painting inside the tank, were exposed to the nitrile to some degree. The extent and duration of their exposure to acetonitrile during the operation were not specified.

One worker complained of chest pains and vomited, coughed, and expectorated blood. He developed convulsions and deep coma during the night and died the following morning. Two workers became seriously ill and were hospitalized the following morning; one of these workers was confused and lethargic. These three employees had worked together in brushing the primer resin paint on the inside of the tank. High concentrations of inorganic cyanide were present in the blood and urine of all three, but actual values were not reported. Thirteen other workers reported to a hospital; five were released after examination, and eight were admitted for overnight observation. No signs or symptoms for the eight workers were mentioned.

Amdur [32], in 1959, studied further the incident of exposure to acetonitrile reported by Grabois [31]. According to Amdur, the corrosion-resistant material consisted of the following four components: (1) a phenolic resin primer; (2) a catalyst containing diethylene triamine, sodium polysulfide (Thiokol), and 30-40% of acetonitrile; (3) a thinner containing 90-95% of acetonitrile; and (4) mica. After 1 day of attempted hand brushing of a mixture of these compounds on the walls of the tank, a modified procedure was adopted for the following days. The primer was heated to 25 C outside the tank before being mixed with the thinner, the catalyst, and a small amount of mica. Positive airflow through the tank was eliminated entirely, and exhaust air was operative for only 45 minutes late in the afternoon on the 2nd day of the operation. The worker who died had worked inside the tank for about 12 hours. Of the two other seriously affected workers, one had worked inside the tank for "about three hours...late in the afternoon," and the other had spent most of a 12-hour workday painting around the ports from the outside of the tank but had painted inside the tank for the last hour of the day. Two other men, less severely affected, had painted inside the tank for no more than 2.5 hours each on the 2nd day. Mixing the paint, sand blasting, and other activities in the work area for varying periods of time on the 2nd day accounted for the exposures of the 10 other workers who were evaluated. Following the

incident, the paint ingredients were not heated, adequate ventilation was provided, and the concentration of organic cyanide was kept below 17 ppm. There were no further incidents.

All workers who reported symptoms were affected within 3-12 hours after exposure on the 2nd day [32]. Their symptoms included chest pain, feelings of tightness in the chest and abdomen, palpitations, shortness of breath, nausea, vomiting, abdominal cramps, urinary frequency, headache, difficulty in swallowing, lassitude, and fatigue. One described his symptoms as being like those of "zinc chills." In a few cases, symptoms persisted for up to a month after the incident, and, in one case, urinary frequency was a continuing complaint. There were no preemployment physical data, but the authors noted that the condition resulting in complaints about urinary frequency may have preceded the exposures.

Signs observed among the exposed group included pale to ash-gray skin; vesicular eczema on the trunk and extremities; initial rapid pulse followed by slow, shallow, and irregular pulse; subnormal blood pressure; subnormal oral temperature; albuminuria; transient hepatomegaly; diminished deep tendon reflexes; transient paralysis of flexor muscles of the hands; stiff neck; and coma [32]. The author reported that 16 individuals were involved; however, only 15 cases were discussed in the report. Nine of these 15 cases were discussed in detail, and serum thiocyanate concentrations were reported for 6 additional workers.

Six workers, including the one who died, had elevated blood cyanide concentrations ranging from 33 to 970 µg% on the initial examination [32]. In the five survivors, these concentrations of cyanide decreased during the following 2 weeks. Seven of nine surviving workers had serum thiocyanate concentrations ranging from 6 to 23 mg% initially and showed a steady decrease in concentration during the next 48 hours. Six additional workers had initial serum thiocyanate values under 3 mg%. No serum thiocyanate value was reported for the worker who died. Gross post-mortem findings included cerebral, thyroidal, hepatic, splenic, and renal congestion.

Amdur [32] stated that the onset of symptoms was so delayed, by from 3 to 12 hours, as to be inconsistent with the well-known ability of cyanide to rapidly depress tissue oxidation by inhibition of the cytochrome oxidase system. He therefore concluded that acetonitrile could not have been the direct cause of the poisonous effects. The author related the signs and symptoms in these workers to thiocyanate, the detoxification product of cyanide, rather than to acetonitrile. He attributed the delayed response to slow release of cyanide and to its metabolism to thiocyanate. According to Amdur, this may have been due, at least in part, to the fact that the alkyl group of acetonitrile, ie, methyl, was less readily oxidized than higher members of the homologous series. He stressed the importance of safe handling of materials. Grabois [31] earlier had discussed

modifications in the ventilation of the tanks and work practices that reduced exposures. The painting of the tanks was completed without further incident.

Dequidt et al [33], in 1972, described the death of a 19-year-old male photographic laboratory worker after exposure to acetonitrile. The worker had handled acetonitrile in a closed vat for 2 days without incident. However, at the end of the 2nd day, he poured an unknown amount of acetonitrile and boiling water on the floor to clean it. The exact duration of his exposure was not determined. About 4 hours after leaving work and eating his evening meal, he experienced gastric distress with nausea and subsequently vomited during the night. When found the next morning, he was sweating profusely and alternately crying out sharply and lapsing into a comalike state. He was taken to a local hospital where he was given a sedative. Later he was transferred to a regional treatment center. There his heart and breathing ceased; he was revived with cardiac massage and an intracardiac injection of adrenalin. Blood examination revealed "large amounts" of cyanides and thiocyanates. He was given dicobalt tetracemate and hydroxycobalamine, neither of which relieved his symptoms. He died 6 days after the onset of poisoning by acetonitrile.

Blood, urine, and tissues (heart, lungs, liver, spleen, kidneys, brain, pancreas, and bladder were analyzed for acetonitrile and for free and combined cyanide [33]. At 44 µg/100 g of tissue, the spleen contained the largest amounts of free hydrogen cyanide, but only traces were found in the bladder. Combined hydrogen cyanide varied from 81 µg/100 g of tissue in the bladder to 1,112 µg/100 g of tissue in the lungs. The largest amounts of acetonitrile were found in the liver (1,184 µg/100 g of tissue) and kidneys (1,535 µg/100 g of tissue). On the 2nd and 3rd days after exposure, his blood contained free hydrogen cyanide, 112 and 87 µg/100 ml, respectively, and combined hydrogen cyanide, 376 and 1,038 µg/100 ml of blood, respectively. On the 3rd day after exposure, the acetonitrile concentration in the blood was 1,176 µg/100 ml and on the 4th day, the acetonitrile concentration in the urine was 311 µg/100 ml. The combined hydrogen cyanide concentration and free hydrogen cyanide concentration in the urine on the 2nd and 3rd days following exposure were 105 and 460 µg/liter, respectively. The authors found acetonitrile in most examined organs 6 days after poisoning, in spite of a large urinary excretion, and thus concluded that because acetonitrile remains in the body so long and releases cyanide so slowly, antidotes should be given slowly and continuously until the victim revives and the acetonitrile is no longer present in the blood.

In 1974, Dequidt et al [34] provided additional details on the acetonitrile exposure they had described in 1972 [33]. Other abnormalities after exposure were hypersalivation, conjunctivitis, totally suppressed or abnormally low output of urine, abnormally low blood pressure (systolic pressure between 70 and 100 mmHg), and albumin in the cerebrospinal fluid and urine.

In 1959, Pozzani and coworkers [35] studied the effects of acetonitrile vapor on 31- to 47-year-old men who inhaled acetonitrile vapor at 40, 80, and 160 ppm for 4 hours each on three separate occasions. Inhalation exposures took place inside a 7,900-liter chamber with a maximum ambient air temperature of 76 F (24 C) and an exhaust airflow of 1,400 liters/minute. Exposure of three men to acetonitrile vapor at 40 ppm was followed a week later by exposure of two of the same subjects at 80 ppm and, 9 days later, at 160 ppm. Blood cyanide and urine thiocyanate concentrations were measured at various times before and after all three exposures.

At 40 ppm, all three subjects recognized the odor of acetonitrile for the first 2-3 hours of the 4-hour inhalation period and then experienced some olfactory fatigue [35]. Throughout the night, following inhalation of acetonitrile at 40 ppm, the youngest subject reported a feeling of tightness in the chest and a sensation in the lungs similar to that experienced when inhaling menthol. The two older subjects exposed at 80 ppm reported no subjective response. After exposure at 160 ppm, one of the two subjects reported a slight transitory flushing of the face 2 hours after inhalation followed about 5 hours later by a feeling of bronchial tightness. These symptoms did not persist overnight.

Cyanide was not detected in the blood of any of the subjects [35]. Thiocyanate excretion in the urine did not correlate with exposure concentrations. The authors concluded that blood cyanide and urine thiocyanate concentrations were not reliable indicators of morbidity due to exposure to acetonitrile when exposures were at low concentrations for short periods of time.

Dalhamn et al [36], in 1968, reported on the absorption through the oral tissues of volatile and aerosolized components, including acetonitrile, found in cigarette smoke. Sixteen 20- to 65-year-old men and women who smoked from 0 to more than 30 cigarettes daily were studied. A smoke-dosage machine was used to blow cigarette smoke into the subjects' mouths; the smoke was never inhaled. After 2 seconds, the subjects blew the smoke (about 60 mm H_2O pressure) through precooled traps to remove water vapor and to collect the smoke component condensates.

From measurements of the amount of acetonitrile and other volatiles in the smoke that either passed through the mouth before condensation or was condensed directly from the smoking machine, it was possible to determine the amount retained in the mouth as a percentage of the amount initially present in the direct smoke [36]. This was done for each component. Of the smoke volatiles examined, acetonitrile was absorbed to the highest extent (74%). In similar experiments in which the smoke was inhaled, Dalhamn et al [37] found that the pulmonary retention of acetonitrile in smoke was 91 ±4.1%.

In 1962, McKee et al [38] reported that acetonitrile is present in the morning urine of smokers. This was confirmed by mass spectrometric analysis of the gas chromatographic fraction in a composite sample of the urine samples from 40 smokers. Acetonitrile concentrations ranged from 2.2 µg/100 ml urine for those smoking three cigarettes/day to values in excess of 20 µg/100 ml of urine for heavy smokers (up to 2.5 packs/day). An average value of 11.76 µg/100 ml urine was determined for the 40 smokers, with a correlation coefficient of 0.707 between the number of cigarettes smoked and the urinary excretion of acetonitrile. These results indicated that acetonitrile, once absorbed into the body, can be excreted unchanged in the urine. In contrast, the concentration of acetonitrile in a composite sample of the urine of 20 nonsmokers was not sufficient to permit a mass spectrometric analysis. The cigarette-smoking studies in humans by McKee et al and Dalhamn et al [36-38] demonstrated that acetonitrile was absorbed by oral tissues, retained by the lungs, and partly excreted unchanged in the urine.

Thiess and Hey [39], in 1969, described a workplace inhalation exposure to ISOBUTYRONITRILE of a 44-year-old man who was rendered unconscious, with convulsive movements of his upper limbs, while filling a tank. He had a soft and thready pulse, dilated pupils, shallow and gasping breathing, and secretion of viscous, glossy mucus from glands of the oropharyngeal area.

After admission to a hospital, the man's condition worsened and he had tonic-clonic movements of the upper extremities [39]. A powerful clenching of the teeth began, and cold sweat formed on the patient's forehead. He was cyanotic, and blood drawn for blood grouping was dark red. The pulse was small and thready, and its rate was 120/minute. The patient was given an iv injection of 1 mg of norepinephrine and was then treated with amyl nitrite, sodium nitrite, and sodium thiosulfate. The man's cyanotic condition diminished and his pulse became stronger, but he continued to have gasping breath and convulsive movements of the upper limbs. He was then given iv injections of lobeline and phenobarbital. Within the next 5-10 minutes, the patient's condition rapidly improved, and he was given a slow iv infusion of 300 ml of whole blood. Four hours after the initial exposure, the patient was fully conscious, although he remembered nothing after the ambulance entered the plant. During the following days, the patient complained often of headache but recovered gradually. He was able to leave the hospital symptom free on the 14th day after admission.

Two other apparently milder cases of isobutyronitrile inhalation exposure were reviewed by Zeller et al [10] in 1969. Details were not given, except that unknown concentrations of isobutyronitrile vapor reportedly produced headache, dizziness, and vomiting 10-60 minutes after exposure and that the intensity of the symptoms varied with the concentration and duration of exposure.

No reports of effects on humans following exposure to propionitrile or n-butyronitrile were found.

(b) Cyanohydrins

In 1960, Wolfsie [40] described an acute exposure of a 30-year-old worker to 70% aqueous GLYCOLONITRILE, in a chemical manufacturing plant. While the operator was filling 55-gallon drums with the solution, his clothing was contaminated because a leak occurred in the filling line that he held under his arm. Since the operator was unaware of his contact with the solution, the extent and duration of his exposure were unknown. The drumming station where he worked was described as a building equipped with adequate local exhaust ventilation. The operator complained of headache, dizziness, unsteady gait, and a sensation described as "rubbery legs," and he vomited within 5-10 minutes after leaving the operation. He was treated at the plant dispensary where he showered and received an unspecified injection from a physician. During the next 7 hours, he appeared to be pale and bewildered, perspired moderately, and had a pulse rate of 104/minute and a respiration rate of 24/minute. He vomited several times and experienced some memory loss. Later, he spoke irrationally, became increasingly unresponsive, and had an irregular rapid pulse. Supportive therapy included bed rest, intermittent inhalation of one ampule of amyl nitrite, 100% oxygen by face mask, and 30 cc of 25% sodium thiosulfate injected iv. Feeling well and apparently fully recovered, the worker was discharged from the hospital in less than 24 hours, without signs of illness. On his return to work the next day, he was assigned to a different job. He continued to feel weak and nauseated for 5 days after returning to work and to experience some congestion of his pharyngeal mucosa for a longer period of time.

A second acute exposure to glycolonitrile reported by Wolfsie [40] involved a 36-year-old worker who used the same kind of filling line and worked under the same environmental conditions as those just described. In this instance, the operator was aware that a 6- x 12-inch area of his clothing had been wet by an unknown quantity of the glycolonitrile solution during the course of the drumming, but it was almost dry by the end of the operation. In less than 1 hour after removing his clothing and taking a soap and water shower, he began to feel weak and dizzy. He drove home with difficulty. He had an unsteady gait and symptoms progressed to severe nausea, repeated vomiting, and severe vertigo; feeling chilled, he went to bed. When he awoke the next morning, his clothing was wet with perspiration, and he felt weak and "washed out." He had no appetite and still felt weak until he ate later in the day after returning to work.

Even though glycolonitrile appears from this study [40] to present a hazard by skin exposure, the possibility that exposure also occurred by inhalation cannot be ruled out. Although local exhaust ventilation equipment was operating, no evidence was provided to indicate that the system was operating efficiently. Wolfsie made two points in the report that suggest the possibility of concomitant inhalation exposure. First,

the subsequent abnormal finding of congested pharyngeal mucosa in the case of the first operator may be indicative of inhalation exposure. Second, glycolonitrile is odorless. Although neither worker reported detecting any odor, vapor and/or aerosol may have been present.

Sunderman and Kincaid [2], in 1953, reported on two fatalities from exposure to ACETONE CYANOHYDRIN. The first case was that of a plant worker whose clothing was splashed with an unknown quantity of acetone cyanohydrin when a tank overflowed. Three hours later, he complained of nausea and was referred to a hospital. On the advice of a physician, he returned to work, but he again felt nauseated. He became unconscious and convulsive and died 6.5 hours after initial exposure. His death was presumed to be associated with skin, and possibly respiratory, absorption of acetone cyanohydrin, although no autopsy record was available. Other details of the exposure were not reported.

The second fatality also involved a plant worker who purportedly drank an unknown quantity of unidentified alcohol obtained from a recovery tank containing traces of acetone cyanohydrin [2]. He lost consciousness and was given unspecified doses of sodium thiosulfate and sodium nitrite. Although he apparently regained consciousness, he died about 12 hours later. Again, no autopsy record was available. The effects produced by alcohol also may have contributed to the fatality.

Sunderman and Kincaid [2] also described nonfatal cases involving three operators who had dermal exposures to acetone cyanohydrin while packing pumps leading to and from storage tanks. The workers lost consciousness but revived when they were exposed to fresh air and their hands cleaned. They suffered no permanent injuries. The workmen stated that, ordinarily, when their hands were covered with grease, the effects of acetone cyanohydrin were minimal. The authors listed cardiac palpitation, headache, nausea, and vomiting as the effects of mild dermal exposure to acetone cyanohydrin.

In 1955, Krefft [41] cited an incident that occurred during a filling operation in a chemical factory. A glass flask containing acetone cyanohydrin burst, and 19 liters of the liquid splashed on the face and clothing of a 51-year-old worker. Her skin was partially washed, but her contaminated clothing was not removed. She was given milk about 5 minutes after the exposure; she subsequently vomited and became short of breath and unconscious. Ten minutes after the accident, tonic-clonic convulsions occurred. When she entered the hospital, her pulse was absent and Cheyne-Stokes respiration was present. She died 1 hour and 20 minutes after exposure, despite attempts to maintain vital functions. Autopsy findings included a bitter almond odor of the internal organs, particularly the brain; hyperemia of the brain and skin; dilatation of the right side of the heart; dark red blood; hyperemia and moderate edema of the lungs; and moderate gastric irritation.

The author [41] reported that death was caused by extensive skin exposure to acetone cyanohydrin over a period of time. Inhalation of an unknown concentration of free hydrocyanic-acid vapor was mentioned as an added factor based on a finding that the free hydrocyanic-acid content of the acetone cyanohydrin varied between 0.007% and 0.7%.

In 1960, Lang and Stintzy [42] reported an incident of acute occupational exposure to acetone cyanohydrin. A 19-year-old worker at a chemical synthesis plant had a portion of his pants wet with acetone cyanohydrin while he was dismantling a conduit containing residual acetone cyanohydrin. Although the quantity of acetone cyanohydrin to which he was exposed was unknown, it apparently did not exceed 30-40 ml. His skin reportedly had direct contact with his wet clothing for 40-60 minutes and with the dry residue for about 5.5 hours.

About 5 hours after initial exposure, the worker complained of headache, retching, a feeling of painful constriction of the throat, progressive weakness, numbness, and dizziness [42]. When he finished work, he changed his clothes and drove home with no apparent difficulty. Whether he washed or showered before changing clothes was not mentioned. After reaching home, the worker's gait was slow and unsteady; he vomited a small amount of bilelike material once; his headache and dizziness increased; and he retired without eating.

He was brought to a hospital approximately 8.5 hours after initial exposure in a state of deep coma [42]. His entire body was cyanotic and reflexes were absent, and he was short of breath. He underwent a period of transitory trismus and muscular fibrillations, followed by tonic-clonic convulsions. He awoke the next morning fully oriented though still tired and said that he remembered nothing that had happened after retiring on the previous evening. A diagnosis of slow intoxication by hydrogen cyanide was made after a review of a report describing the work clothes the patient had worn on exposure, which retained the characteristic odor of acetone cyanohydrin. The patient remained hospitalized for 10 days. He returned to work nearly a month after the incident.

The authors [42] considered this episode a rare example of delayed hydrogen cyanide poisoning resulting from the dissociation of acetone cyanohydrin into acetone and hydrogen cyanide. The dissociation of acetone cyanohydrin is favored by an alkaline medium, and this was considered to be present in the form of perspiration on the skin. The authors postulated that hydrogen cyanide easily permeated the vascular and lymphatic endothelium by the ability of acetone to dissolve lipid substances in these tissues.

Thiess and Hey [39], in 1969, described an exposure to acetone cyanohydrin involving a 23-year-old man who was filling large drums using a rubber hose connection from a tank car. Apparently this was not the usual

method, but it was employed because of a defective part. The affected worker was not wearing a face mask or rubber overalls, and the gloves he wore were made of cloth rather than rubber. During the operation, one glove got soaked; after completing the job, the man put the wet glove in his trouser pocket. Within 5 minutes he vomited. When he became unconscious 10 minutes later, he was taken to the company's infirmary. His breathing was difficult and irregular, and he was given artificial respiration. He regained consciousness after receiving amyl nitrite but lapsed again into unconsciousness and showed tonic-clonic convulsions of the extremities. After he was treated with sodium nitrite and sodium thiosulfate, his symptoms again subsided temporarily. When the soaked glove, which had served as a continuing source of exposure, was discovered in his trouser pocket, he was bathed and given a second course of sodium nitrite and sodium thiosulfate treatment. He improved quickly; he was hospitalized for 3 days and returned to work 8 days later.

Zeller et al [10], in 1969, described two cases of acetone cyanohydrin skin exposure and one of combined exposure to acetone cyanohydrin and isobutyronitrile but provided no details of exposure or effects. The authors did report that the effects of acetone cyanohydrin poisoning were generally like those of intoxication of isobutyronitrile. Thiess and Hey [39] also commented that, in the cases of acetone cyanohydrin skin exposure and isobutyronitrile inhalation exposure, the illnesses resulting from these two chemically related compounds were quite similar although the routes of exposure were different.

(c) Dinitriles

Hyden and Hartelius [26], in 1948, reported on the clinical use of MALONONITRILE in the treatment of various forms of mental illness. The authors found that administration of malononitrile to rabbits increased the concentration of protein and polynucleotides in slices of brain cortex, spinal cord, paravertebral ganglia, and other areas of the CNS examined by UV absorption spectroscopy. Using the same spectroscopic method, Hyden and Hartelius found that pyramidal cells of the frontal cortices of psychiatric patients contained less protein and nucleic acid than those of accident victims. Malononitrile was subsequently administered to 66 patients, most diagnosed as schizophrenic or depressed, as an experimental treatment based on the rationale that malononitrile might restore the cellular function by stimulating the production of protein and polynucleotides within the nerve cells. Fasted individuals were given malononitrile 5% solution iv during the course of treatment at doses ranging from 1.0 to 6.0 mg/kg of body weight. The number of doses given each patient ranged from 3 to 17. Infusions were given, on the average, 2 or 3 times a week with at least 1-day intervals. Each treatment lasted from 10 to 69 minutes. Tachycardia occurred 10-20 minutes after infusion of malononitrile in every case. Facial redness, headache, nausea, vomiting, shivering, cold hands and feet, muscle spasms, and numbness also were reported with varying frequency.

Convulsions were seen in two patients, neither of whom had a history of epilepsy, and cardiac collapse occurred in one patient with a congenital heart defect.

MacKinnon et al [27], in 1949, described side effects similar to those described by Hyden and Hartelius [26] in nine psychiatric patients given 2-4 mg/kg doses of malononitrile as a 5% solution. Patients received 10 iv administrations over a 2- to 3-week period. Toxic effects occurred 15 minutes after the injection. In contrast to the side effects reported by Hyden and Hartelius, no convulsions occurred in the group of patients; however, feelings of nausea often reappeared several hours after treatment.

In 1950, Hartelius [29] reported treating 40 psychiatric patients with malononitrile (5% solution). Doses averaging 2.4 mg/kg body weight were administered iv. Each patient received from 3 to 12 injections in 24 days. The average duration of each treatment was 48 minutes. Facial redness, tachycardia, and congestive flow of blood to the head were consistently observed throughout the treatment.

Meyers et al [28], as reported in 1950, treated six psychiatric patients with malononitrile (5% solution) at doses of 3-6 mg/kg of body weight administered iv. The patients, who were fasted before each treatment, were given infusions during a 21- to 60-minute period, with an average of about 30 minutes. Reactions during treatment included: flushing of the face, appearing 5 minutes after treatment began and increasing throughout treatment; onset of tachycardia in 10-15 minutes; feelings of nausea in 20-25 minutes; and vomiting in about 30 minutes. Patients became restless and acutely distressed. Veins of the head and neck became distended, and extremities were cold. In some cases, there was an increase in systolic blood pressure and a decrease in pulse pressure. The authors indicated that the dose at which toxic effects were produced and the time of their onset varied with the individual.

In 1955, Ghiringhelli [16] reported a case of acute accidental poisoning in which an 18-year-old man drank a few cc of ADIPONITRILE while at work. About 20 minutes after ingestion, he vomited and experienced tightness in the chest, headache, profound weakness with difficulty standing, and vertigo. On admission to the company infirmary, he was observed to be cyanotic. He had rapid heartbeats, rapid and raspy respirations, and a low blood pressure. His pupils were dilated and barely reacted to light. Tonic-clonic contraction of limb and facial muscles and mental confusion were also present. Initially, his stomach was pumped out; but when the symptoms did not subside, single doses of 15 cc of 25% sodium thiosulfate and 20 cc of 40% glucose were injected iv. The signs and symptoms subsided within 10 minutes after treatment began, and he appeared fully recovered for about 4 hours. Then his illness recurred and continued in greater severity for at least 2 hours. Following a second course of treatment with sodium thiosulfate and glucose, the patient slowly and completely recovered.

Zeller et al [10], in 1969, reviewed seven cases of skin exposure to adiponitrile. Six of the seven cases resulted in skin irritation and inflammation 5-15 minutes following exposure, but none required hospitalization. A seventh worker suffered extensive destruction of the skin of one foot after his shoe was drenched with adiponitrile. He required surgical treatment and was incapacitated for 117 days. No details of exposure or clinical manifestations were discussed for any of the cases.

In 1957, Reinl [43] reported on a study of 16 workers who were allegedly suffering ill effects from exposure to the vapor of TETRAMETHYLSUCCINONITRILE at an unknown concentration. The investigation began after five cases of convulsions and unconsciousness occurred during an 18-month period at one plant where workers used azo-isobutyronitrile as the propellant gas to produce polyvinyl chloride foam.

The workers, seven women and nine men, either had cut and welded slabs of newly expanded foam or operated presses and mixers, within poorly ventilated areas [43]. Their ages ranged from 18 to 58 years and their length of service from 2 months to 5 years. The tetramethylsuccinonitrile was released by thermal decomposition of the azo-isobutyronitrile. The study consisted of detailed medical histories and a physical examination, with a limited number of laboratory tests on only 3 of the 16 subjects. The results of the physical examinations and laboratory tests (serum protein electrophoresis and limited liver function tests) were inconclusive, and no characteristic or consistent abnormalities were noted.

The signs and symptoms reported at the time of the investigation [43] included headache (by 4) or a sensation of pressure within the head (by 12), dizziness (by 5), nausea (by 7), vomiting (by 5), a peculiar taste (by 3) and frothy spittle in the mouth (by 7), respiratory distress (by 4), fatigue (by 3), unconsciousness (by 2 women), and convulsions (by 5). Complaints were more prevalent among workers involved in the cutting, thermal welding, and storage of the foam than among press and mixer workers.

All signs and symptoms of overexposure subsided following the installation of improved ventilation in the work areas. All 16 workers were medically checked every 14 days for the year following the original investigation, and no further symptoms were found.

No environmental measurements of tetramethylsuccinonitrile or of any other airborne contaminant were reported. Since the workers were exposed not only to tetramethylsuccinonitrile but also to a number of other chemicals including vinyl chloride monomer and azo-isobutyronitrile, it is not possible to definitely ascribe the reported effects of exposure to tetramethylsuccinonitrile alone.

Epidemiologic Studies

No epidemiologic studies of workers at risk from selected nitriles were found in the literature.

Animal Toxicity

The animal toxicity data for individual species by various routes of administration for mononitriles, cyanohydrins, and dinitriles are presented in Table III-3. The LD_{50} values for mice, rats, guinea pigs, and rabbits are shown in Table III-4. Szabo (S Szabo, written communication, May 1978), using female rats (200 g) of the Sprague-Dawley-derived Charles River CD strain, determined approximate LD_{50} values for adiponitrile, n-butyronitrile, isobutyronitrile, propionitrile, malononitrile, and succinonitrile. These LD_{50} values by sc and ip administration are shown in Table VI-1 and provide a basis for quantitative comparisons of toxicity for some of the selected nitriles. The toxicities to animals of mononitriles, cyanohydrins, and dinitriles are discussed below.

(a) Mononitriles

Smyth and Carpenter [44], as reported in 1948, exposed Sherman rats, in groups of six each, to ACETONITRILE administered orally or by inhalation. Doses differing by a factor of 10 were used to estimate an LD_{50} value by oral administration, and exposure to acetonitrile at 8,000 ppm for 4 hours was used to assess the effects of inhalation. To evaluate dermal toxicity, the authors kept the clipped skin of rabbits in contact with acetonitrile for 24 hours by means of a rubber cuff surrounding the animals' abdomens.

Primary skin irritation and eye injury resulting from contact with acetonitrile were described as being comparable to that resulting from acetone exposure [44]. The single dose oral LD_{50} for acetonitrile was estimated to be 3.8 g/kg in Sherman rats. One of six rats died after inhaling acetonitrile at 8,000 ppm for 4 hours. The percutaneous LD_{50} for acetonitrile in rabbits was 3.9 g/kg.

In 1971, Kimura et al [45] presented the results of their studies concerning the effect of age on the acute oral toxicity of acetonitrile in rats. Test animals were newborn Sprague-Dawley rats (24-48 hours old, weighing 5-8 g), 14-day-old rats (weighing 16-50 g), young adult rats (weighing 80-160 g), and older adult rats (weighing 300-470 g). Analytical grade acetonitrile was administered orally via straight needle in undiluted form, and the nonfasted rats were observed for a week.

Observable signs of toxic action in the rats ranged from labored breathing to ataxia, cyanosis, and coma [45]. The acute oral LD_{50}'s for the 14-day-old, the young adult, and the adult rats were 0.16 g/kg, 3.1

g/kg, and 3.5 g/kg, respectively. Acetonitrile was significantly more toxic in the 14-day-old than in the adult rats ($P < 0.05$). The lowest dose of acetonitrile that produced any sign of toxicity in young adult rats was 1.6 g/kg. An accurate assessment of the LD_{50} of acetonitrile in newborn rats was not possible because of their extreme sensitivity to the compound.

In 1959, Pozzani et al [35] summarized investigations of the toxicity of acetonitrile administered by various routes to mice, rats, guinea pigs, rabbits, dogs, and monkeys. Twelve separate acute oral toxicity tests were performed over a 5-year period on an unspecified number of male and female rats (Carworth Farms-Wistar or Nelson albino strains), weighing 30-425 g. Acetonitrile was diluted in corn oil, water, or 1% aqueous Tergitol 7 for administration by gastric intubation. The LD_{50} values ranged from 1.3 to 6.7 ml/kg; male rats were about three times as susceptible as females. Guinea pigs, rabbits, dogs, and monkeys were similarly tested, and the LD_{50} values for the various species and routes of administration are given in Table III-2.

When two groups of six Carworth Farms-Wistar rats were exposed to acetonitrile vapor at approximately 53,000 ppm, three of six rats exposed for 30 minutes died and none of the rats exposed for 15 minutes died [35]. Twenty groups of 12 male or 12 female rats were exposed for 4 or 8 hours to acetonitrile vapor at concentrations ranging from 1,000 to 32,000 ppm. Calculated LC_{50}'s for the 8-hour exposures were 7,551 ppm (males) and 12,435 ppm (females); the 4-hour LC_{50} was 16,000 ppm (males and females). The 4-hour LC_{50}'s determined in three groups of six (male and female) guinea pigs each and three groups of four male rabbits each were 5,655 and 2,828 ppm, respectively. Exposure of three dogs for 4 hours at 16,000 ppm and above killed all dogs, whereas a 4-hour exposure at 8,000 ppm and below killed none of three dogs. Most resistant to acetonitrile vapor were the rats, followed in decreasing order by dogs, guinea pigs, and rabbits. Although male rats appeared to be more susceptible than females to 8-hour inhalation exposures to acetonitrile, no sex difference was observed in the 4-hour exposures.

Groups of 15 male and 15 female Carworth Farms-Wistar rats weighing about 140-200 g were exposed 7 hours/day, 5 days/week, for a total of 18 weeks, to acetonitrile at 166, 330, or 655 ppm [35]. Acetonitrile concentrations were checked four times/day with a portable Zeiss interferometer. Four groups of 15 male or 15 female rats under similar experimental conditions, but without exposure to acetonitrile, served as controls. No deaths and no significant differences between the test and control groups in growth rates or in relative liver and kidney weights were seen. Microscopic examination showed that, of the 28 rats inhaling 166-ppm vapor, 1 had macrophage clumps in the alveoli and another suffered lung collapse. Of the 26 rats inhaling 330-ppm vapor, only 3 rats showed lung abnormalities including bronchitis, pneumonia, atelectasis, and macrophage clumps in the alveoli. Rats that inhaled 655-ppm vapor had lung, kidney,

and liver damage. Also found were transitory lesions, such as alveolar capillary congestion and focal edema, often accompanied by bronchial inflammation, desquamation, and hypersecretion of mucus; cloudy swelling in kidney tubules; and central reversible osmotic swelling of mitochondria of liver cells.

Urine samples pooled (59-62 days) from the rats exposed to acetonitrile at 166 or 330 ppm were analyzed for thiocyanate [35]. Samples showed thiocyanate levels of 17-79 mg/100 ml, but there was no direct correspondence with exposure level. However, after a 3-day rest, the urine samples from both groups were free of thiocyanate.

Four rhesus monkeys were exposed to acetonitrile at 330, 660, or 2,510 ppm 7 hours/day, 5 days/week, for up to 99 days [35]. Three of the monkeys had received acetonitrile and sodium thiocyanate iv 3 months earlier, and one did not. None of the four monkeys had appreciable weight loss during the inhalation periods. The monkey exposed at 2,510 ppm appeared normal after the 1st day's exposure but died on the 2nd day during reexposure, following labored breathing and prostration. Autopsy revealed engorgement of the dural capillaries and pleural effusion. Two monkeys exposed at 660 ppm appeared normal for the 1st week of exposure, but they began to show poor coordination during the 2nd week. One of the monkeys died on the 23rd day of inhalation. The other died on the 51st day of exposure. The monkey exposed to acetonitrile at 330 ppm was killed after showing hyperextension reflexes and hyperexcitability toward the end of the 99-day exposure period. The three monkeys that died during the exposure period had dural venous sinus hemorrhages and occasional fibrous tissue proliferation in the lungs.

Three male rhesus monkeys and three male dogs were exposed to acetonitrile at a nominal concentration of 350 ppm for 7 hours/day, 5 days/week for 91 days [35]. A significant decrease in the mean body weight of the dogs was observed on 10 occasions during the first 72 days of the study, whereas no striking weight changes were seen in the monkeys. Other changes included a depression of the hematocrit and hemoglobin values of the dogs during the 5th week, whereas the erythrocyte count of one of the monkeys was significantly increased throughout the study. At autopsy, all monkeys showed slight to moderate hemorrhage in the dural venous sinuses. Microscopic examination of lung tissues showed focal emphysema, as well as fibrous tissue and macrophage proliferation. The dogs showed no gross macroscopic changes. Microscopic examination revealed some focal emphysema and alveolar septal proliferation.

Blood samples from the monkeys contained 4.7-5.4 µg of cyanide/100 ml following a 5-consecutive-day inhalation period and 1.8-2.9 µg/100 ml after 2 days of no exposure [35]. The dogs showed 7.6-9.2 µg cyanide/100 ml following a 5-day inhalation period, and no detectable cyanide after a 2-day rest. Both dogs and monkeys excreted thiocyanate in the urine during

exposure to acetonitrile, and detectable amounts were still present 2 days postexposure.

To measure how much cyanide was formed during inhalation of lethal amounts of acetonitrile, the authors [35] exposed three dogs at 16,000 ppm for 4 hours. Blood cyanide levels reached a peak of 305-433 (u)g/100 ml after 3 hours of exposure and then decreased during the final hour. All three dogs died within 14 hours after exposure.

Pozzani et al [46], in their comparison of the oral and inhalation toxicities of equivolume mixtures of several industrial chemicals, first determined single-dose oral LD_{50}'s and single 4-hour inhalation LC_{50}'s for each of seven chemicals: acetonitrile, acetone, dioxane, ethylacetate, carbon tetrachloride, toluene, and propylene oxide. Groups of six female Carworth Farms-Nelson rats of unspecified weights and age were used. For the oral LD_{50} determination, the rats in each group received single oral doses of undiluted acetonitrile or an equivolume mixture of acetonitrile and one of the chemicals in each of six tests. For the LC_{50} determinations, the rats were allowed to inhale vapor of acetonitrile alone or in a 50:50 vapor mixture with each of the six other chemicals, in turn, for 4 hours.

For acetonitrile, the experimental oral LD_{50} was 6.5 g/kg, and the single 4-hour inhalation LC_{50} was 26.9 mg/liter [46]. Of the mixtures tested, only the acetonitrile-acetone administration had a tendency to yield more than additive effects by inhalation (LC_{50}, 14.6 mg/liter) and by oral administration (LD_{50} = 2.2 g/kg), as compared with expected values of 39.7 ml/liter and 9.99 ml/kg, respectively. All the other chemical pairs including acetonitrile produced essentially additive effects when administered either orally or by inhalation. Smyth et al [47] also determined that an equivolume mixture of acetone and acetonitrile given orally to rats was more acutely toxic than would have been expected had their toxicities been additive.

In 1932, Marine et al [48] studied the production of goiter and exophthalmos in prepubertal rabbits following sc administration of acetonitrile for up to 63 days. Male and female rabbits of Dutch and Belgian breeds, aged 3-5 months and weighing 1,184-1,911 g, were given daily injections of 79-118 mg/cc of acetonitrile. Exophthalmos developed as early as day 20 in the 3-month-old rabbits that received daily injections of 79 mg of acetonitrile. According to the authors, this effect was seen only in the young Dutch rabbits and did not occur at all in the adult rabbits (6 months and older) of either strain.

Marine et al [48] noted a close relationship between exophthalmos and thyroid hyperplasia. Exophthalmos was absent in rabbits that showed little or no thyroid hyperplasia. When hyperplasia was more intense, exophthalmos appeared and was said to be proportional to the degree of hyperplasia.

Exophthalmos is commonly a symptom of hyperthyroidism, but the authors did not speculate as to whether its occurrence was a direct or thyroid-mediated effect.

In 1932, Spence and Marine [49] investigated the production of thyroid hyperplasia in rats given acetonitrile in small doses. Twelve female albino rats, six litter mates aged 3 months and six litter mates aged 5 months, were divided into three groups, two rats from each litter. The animals were fed a nongoitrogenic diet and received daily sc injections of acetonitrile in water at doses of 0.08 cc (62.4 mg), 0.04 cc (31.2 mg), and 0.02 cc (15.6 mg).

At the end of 21 days, one rat from each group was killed [49]. At autopsy all animals showed only slight thyroid hyperemia. After 28 days of treatment, the rats showed definite thyroid hypertrophy. During the next 8 days, the daily doses were gradually increased for the remaining nine rats until those initially on the smallest dose were receiving 0.05 cc (39 mg), and those initially on the largest dose were receiving as much as 0.15 cc (117 mg) of acetonitrile daily without any sign of cyanide poisoning. After 36 days of treatment, the thyroids were larger with increased hyperemia. In general, these changes were proportional to dose.

A similar study in mice was carried out by Spence and Marine [49]. Twelve mice, 3.5 weeks old, weighing an average of 13 g and on the same diet as the rats, were divided into three groups to receive daily sc injections of acetonitrile at doses of 0.005 cc (3.9 mg), 0.0025 cc (1.95 mg), and 0.00125 cc (0.975 mg). After 11-34 days, only a slight thyroid reaction was produced. From these results, the authors concluded that, because thyroid reactions obtained in rats and mice exposed to acetonitrile were far less than those reported [48] in rabbits receiving much smaller doses, rats and mice possess considerable resistance to goitrogenic substances.

In 1927, Crivellari [50] reported that removal of the adrenal glands of white rats resulted in a hundredfold increase in sensitivity to acetonitrile injected sc. In 1934, Degti [51] found that removal of the suprarenal gland capsules of albino rats resulted in a twofold increase in sensitivity to acetonitrile injected sc.

Dessau [52], in 1935, described the results of his investigations of the protective function of the adrenal gland in acute acetonitrile poisoning in rats. Acetonitrile, at a dose of 5.0 mg/g, was administered intraperitoneally (ip) to 164 adrenalectomized rats weighing 50-150 g. Ninety of the rats had received adrenal implantations in the peritoneal cavity or on the ovaries. At 24 hours after the acetonitrile injection, only 8 of the 74 nonimplanted rats were still alive, whereas 19 of the 90 adrenal-implanted animals survived. Microscopic examination of the implantation sites in the surviving rats showed only altered residues of

adrenocortical substance. In a separate experiment, epinephrine at 10 and 100 µg was said to be without influence on the acetonitrile resistance of nine rats, but no actual results were cited. In yet another experiment, adrenalectomized rats were reportedly one-sixth less resistant to acetonitrile (tested 4-5 days after surgery) than control rats. No further experimental details were provided for these observations. The author concluded that the adrenal gland protects against acetonitrile poisoning and that the resistance-increasing factor is associated with the adrenal cortex rather than the medulla.

In 1972, Dequidt and Haguenoer [53] described their preliminary investigation to determine the distribution, metabolism, and excretion of acetonitrile in rats, in order to find the best antidotes and treatment in humans who had been overexposed to this compound. Furthermore, they wanted to define safe limits for acetonitrile exposure in the occupational environment.

Initially, two groups of four rats each and one group of three rats were given a single ip injection of 780 mg/rat (average weight 330 g) [53]. All of the animals died in 3-12 hours. The liver, lungs, spleen, kidneys, heart, brain, muscle, intestines, stomach, testes, and skin of each animal were analyzed for acetonitrile and both free and combined hydrogen cyanide content. At 359 µg/100 g tissue, combined hydrogen cyanide concentration was lowest in the liver; the concentrations in the spleen, stomach, and skin were 1,347, 1,757, and 1,045 µg/100 g of tissue, respectively. Free hydrogen cyanide found in the organs varied from 17 µg/100 g of tissue in the liver to 347 µg/100 g for the spleen. Acetonitrile was found to be evenly distributed in various organs.

Haguenoer and colleagues [54], in 1975, reported on the distribution and metabolic fate of acetonitrile in white male Wistar rats. Each rat (housed in groups of three) was injected ip with acetonitrile at single doses of 2,340, 1,500, or 600 mg/kg. Rats given the two highest doses died as a result of exposure, whereas rats given 600 mg/kg survived with no apparent signs of toxicity but were killed for autopsy on the 11th day. The heart, lungs, liver, spleen, kidneys, stomach, intestines, skin, muscle, brain, and testes of each animal were examined for acetonitrile and for free and combined hydrogen cyanide. The combined hydrogen cyanide consisted essentially of thiocyanates, plus cyanohydrins and cyanocobalamine.

On each of the 11 days postexposure, urine was collected from the 600 mg/kg rats for measurements of free and combined hydrogen cyanide and acetonitrile [54]. On day 1, the urine contained an average of 92 µg free hydrogen cyanide, 5,391 µg combined hydrogen cyanide, and 20.3 mg acetonitrile. No acetonitrile was measured after day 4, and free hydrogen cyanide excretion averaged 5.3 µg/animal on day 11. Each control rat excreted from 1.5 to 5.2 µg of free hydrogen cyanide and from 9 to 40 µg

combined hydrogen cyanide each day. No acetonitrile was found in the urine of control rats at any time. Tissue analyses at autopsy showed no important differences between the treated and the control rats. There was a dramatic decrease in the excretion of both forms of hydrogen cyanide after day 4 when acetonitrile was no longer present in the urine.

The authors [54] concluded that acetonitrile was low in toxicity and that the amount of the cyanide ion present was dependent on the rate of release of cyanide from the parent molecule. Also, they postulated that the large amounts of hydrogen cyanide liberated at the high doses (2,340 and 1,500 mg/kg) were responsible for the rat deaths.

In 1975, Haguenoer et al [55] reported their observations on the distribution and metabolic fate of acetonitrile in the rat after inhalation of acetonitrile at 2,800 or 25,000 ppm. At 25,000 ppm, all three rats died 30 minutes after the start of the exposure, following difficult breathing and cyanosis. Chemical analysis of various organs (heart, lungs, liver, spleen, kidneys, stomach, intestines, skin, muscle, brain, and testes) were made. The mean concentrations of acetonitrile ranged from 136 to 2,438 µg/100 g of muscle and kidney, respectively, and of free hydrogen cyanide, from 27 to 402 µg/100 g of liver and spleen, respectively. The free hydrogen cyanide was more uniformly distributed, except in the spleen (402 µg/100 g) and in the brain (129 µg/100 g), where it was somewhat higher.

The authors [55] stated that the high concentrations of acetonitrile (2,438 µg/100 g) found in the kidneys may have been due to either very high excretion of the acetonitrile or renal blockage. Acetonitrile concentrations in all the organs of rats exposed by inhalation (25,000 ppm) were up to 16 times those observed in a similar ip study [54]. In contrast to the latter study in which ip administration of acetonitrile was associated with a latency period of 3-12 hours between dosing and death, the rats in the present study died immediately after inhalation.

In the second experiment, three rats inhaled acetonitrile at 2,800 ppm, 2 hours/day for up to 5 days [55]. All showed labored breathing, temporary anuria, and diarrhea. After the third exposure, one rat died with lung and brain hemorrhages. After the fourth exposure, the remaining two rats suffered paralysis and decreased urinary excretion. One died at the start of the fifth exposure, and the other died 2 hours after the exposure was completed. Both rats had lost about 45% of their body weight during 5 days of exposure. Autopsies of the rats revealed that all the organs examined contained concentrations of acetonitrile and free hydrocyanic acid in the range of 96.0-286.9 and 53-990 µg/100 g tissue, respectively. Organ concentrations of acetonitrile were high but variable in the three animals (highest in the kidneys, 286.9 µg/100 g tissue). These values were lower than those noted for the 25,000-ppm intoxications. The authors attributed this to a greater pulmonary elimination (exhaled air) of the acetonitrile between exposures. By comparison, the average organ concentrations of free

hydrocyanic acid were slightly higher than those for the 25,000-ppm group, particularly in the spleen (990 µg/100 g tissue). However, the relative increase was greatest in the heart (4.9 times) and stomach (5.6 times) compared with only 2.4-fold in the spleen.

The authors [55] stated that the organ concentrations of hydrogen cyanide in the animals that died from inhaling acetonitrile were similar to those found in the animals that died from ip doses of acetonitrile. Additionally, the results implied that there was no quantitative relationship between the organ concentrations of the free hydrogen cyanide and exposures to acetonitrile. At either concentration of acetonitrile, a lengthy and persistent anuria was always observed as one of the effects. Such signs varied with the amount of acetonitrile inhaled and with the sensitivity of the animal.

In 1972, Szabo and Selye [56] reported the ulcerogenic effect of PROPIONITRILE in female rats. Forty test animals were divided into 4 groups of 10 rats each. Twenty animals, 10 each with a mean body weight of either 200 or 100 g, were administered sc doses of propionitrile three times daily for 4 days. The daily dose was increased from 6 mg/100 g body weight on day 1 to 8 mg/100 g on day 2, 15 mg/100 g on day 3, and 20 mg/100 g on day 4. Twenty animals, 10 each with a mean body weight of 100 or 200 g, were given sc doses once a day for 4 days. The daily dose was increased from 15 mg/100 g body weight on day 1 to 20 mg/100 g on day 2, 40 mg/100 g on day 3, and 50 mg/100 g on day 4. Duodenal tissues from rats that died or were killed with overdoses of chloroform were prepared for microscopic examination. Other unspecified organs were examined for gross and microscopic changes.

Szabo and Selye [56] observed that female rats given propionitrile in single or multiple sc injections at 6-50 mg/100 g body weight for 4 days developed duodenal ulcers, often perforating, on the 4th or 5th day of the experiment. The ulcers developed on the antimesenteric mucosal surface of the duodenum about 5-8 mm caudal to the pylorus. An unstated number of rats administered propionitrile died on day 2 of exposure. Examination of the duodenum of these animals showed no occurrence of duodenal ulcers.

Eight of 10 rats (mean body weight of 200 g) that were administered propionitrile sc in doses of 6 mg/100 g, 8 mg/100 g, 15 mg/100 g, and 20 mg/100 g of body weight three times a day for 4 days developed lesions of the duodenal mucosa [56]. Four of these eight rats had deep erosions in the duodenum, which involved the muscularis mucosae in some animals. The four remaining rats developed perforation of the duodenum with subsequent ulcer penetration into the liver. One rat also showed a perforated gastric ulcer, accompanied by peritonitis. Four of 10 rats (mean body weight of 200 g), administered propionitrile sc in doses of 15 mg/100 g, 20 mg/100 g, 40 mg/100 g, and 50 mg/100 g of body weight once a day for 4 days, subsequently developed duodenal ulcers. One of the four animals had a

perforated duodenum and accompanying peritonitis. In groups (mean body weight of 100 g) treated once or three times daily, one rat in each of the single- and multiple-dose studies developed duodenal ulcers. No structural changes in other body organs (not specified) were observed following propionitrile administration; however, evidence of lung edema was reported in animals that received unspecified doses of propionitrile. Rats administered propionitrile developed prostration and dyskinesia (number of animals and dosage not specified). Mortality was 80-100% in the treated animals. Most of the rats died by the 4th day of dosing.

Szabo and Selye [56] concluded that susceptibility to the ulcerogenic potential of propionitrile was age and dose related. Adult rats (200 g) were more likely than young ones (100 g) to develop duodenal ulcers. Also, rats administered propionitrile once a day for 4 days showed a lower incidence of duodenal ulcers than did those rats receiving a similar dose three times a day. Szabo et al [57] reported similar results in 1977 as part of their study on the influence of propionitrile on gastric acid secretion in female rats.

In 1975, Giampaolo et al [58] reported on the cellular effects of propionitrile in the stomach and duodenum in female rats. Rats weighing 200 g were administered either a single sc dose of 6 mg/100 g body weight of propionitrile or two doses 3 hours apart. The animals were killed 2 hours following the single dose or 5 or 8 hours after the injection of the two doses. Portions of tissue from the stomach and duodenum were fixed in Karnovsky's fixative by luminal or aortic perfusion and prepared for examination by electron microscopy.

The authors [58] found that propionitrile induced structural changes in the cells of the duodenum and stomach in female rats. Morphologic changes observed in the duodenal cells included vacuolization and alteration of the nuclear chromatin pattern, disarray and clubbing of the microvilli, and a progressive necrosis of cells down the sides of the villiferous folds. Giampaolo et al also observed a dilation of the intracellular canaliculi in the acid-producing parietal cells of the gastric mucosa 5 hours following two injections of propionitrile. Structural changes in the duodenum were not reported 2 hours after a single injection. These findings generally concur with Szabo and Selye's earlier report [56] of propionitrile-induced duodenal lesions in rats.

Dzau and associates [59], in 1975, reported that the incidence and intensity of propionitrile-induced duodenal ulcers in female rats were reduced by 50% following administration of metiamide, a histamine antagonist. These findings are consistent with another report [60] of a reduction in ulcer formation following treatment with gastric antisecretory agents.

Haith and coworkers [61], in 1975, described preliminary investigations of the effects of bilateral vagotomy and hypophysectomy in female rats

treated with propionitrile. They reported that bilateral vagotomy completely inhibited the occurrence of duodenal ulcers in rats and that hypophysectomy significantly reduced the incidence and severity of duodenal lesions. The authors concluded that the CNS affected the ulcerogenic property of propionitrile.

In 1975, Robert et al [60], in a report on factors that influence the ulcerogenic effects of propionitrile in rats, observed a decrease in the frequency of duodenal ulcers in rats that were fasted throughout the experiment, as compared with those that were allowed to eat at will. Male rats were more resistant than females to the induction of duodenal ulcers by propionitrile; the incidence in males was 15% vs 80% in females. There appeared to be no significant differences among the toxicities of propionitrile administered by various routes.

In a second series of experiments, the occurrence of duodenal ulcers was prevented by methscopolamine bromide and 16,16-dimethyl prostaglandin E_2 [60]. The effects of these agents were dose dependent. Prednisolone increased the toxicity of propionitrile in rats: 83% of the animals administered prednisolone sc along with propionitrile died as compared with 8% of those administered only propionitrile (60 mg/kg sc twice a day). Propionitrile-induced ulcers were not significantly affected by adrenocorticotropin but, at the highest dose (12 USP units), the mortality decreased from 42 to 8%. Desoxycorticosterone did not significantly influence the toxicity or ulcerogenicity of propionitrile in rats.

In 1975, Szabo and Reynolds [62] reported on the ulcerogenic effect of propionitrile. Female Spraque-Dawley rats (200 g) were administered sc doses of propionitrile. The doses given were 60 mg/kg, 80 mg/kg, 100 mg/kg, and 100 mg/kg three times per day on days 1, 2, 3 and 4, respectively. All animals died as a result of exposure before the 5th day. The authors reported that 80% of the rats had developed duodenal ulcers, but they observed no adrenal necrosis. Szabo and Reynolds considered propionitrile and some structurally related compounds to be potent ulcerogens in rats.

Haguenoer et al [63], in 1974, published animal studies of n-BUTYRONITRILE. Eighteen male rats of unspecified strain were divided into four exposure groups of three rats and one exposure group of six rats. All rats in the first four groups were administered a single ip dose of pure n-butyronitrile and were observed until death. Autopsies were performed; and tissues from the heart, lungs, liver, spleen, kidneys, stomach, intestines, skin, muscle, brain, and testes were examined to determine average concentrations of n-butyronitrile and free and combined hydrogen cyanide.

The first group of three rats (average weight 260 g) received single ip doses of 1,440 mg/kg [63]. The rats became comatose and cyanotic, and all

died within 90 minutes. Evidence of cerebral hemorrhages was seen in one rat at autopsy. All organs contained n-butyronitrile, free hydrogen cyanide, and combined cyanides. The highest average concentration of n-butyronitrile was found in the lungs, whereas the highest free hydrogen cyanide concentrations were in the heart and brain.

The second group of three rats (average weight 323 g) received n-butyronitrile in single ip doses of 600 mg/kg [63]. The rats became convulsive, short of breath, and comatose; and all died within 75-120 minutes after exposure. Two rats also had excess salivation. Except for the lungs, where the average n-butyronitrile concentration was only about 20% of the average 1,440-mg/kg dose, organ concentrations of n-butryonitrile averaged about 50% less. Free hydrogen cyanide was present in all organs and at the same order of magnitude as found at doses of 1,440 mg/kg. Combined hydrogen cyanide was present in all organs at lower average concentrations than those found at doses of 1,440 mg/kg, except that in the brain it was almost twice as great.

The third group of three rats (average weight 300 g) received single ip doses of 300 mg/kg [63]. In this group, respiration rate increased, and the animals became comatose before death occurred 12-14 hours after injection. n-Butyronitrile was present in all organs examined, but at lower average concentrations than at previous doses (one-third to one-eighth of those found at doses of 600 mg/kg). Free hydrogen cyanide was present in all organs at average concentrations close to those found at both previous doses. Combined hydrogen cyanide average concentrations were two to eight times greater than those found at both previous doses.

The fourth group of three rats (average weight 285 g) received single ip doses of 150 mg/kg [63]. The rats suffered nasal hemorrhages, and cyanosis preceded their deaths 21 hours after exposure. As in the other groups, n-butyronitrile was present in all organs examined at lower average concentrations than those found at previous doses. Average concentrations of both free and combined hydrogen cyanide showed a general increase over those found at doses of 300 mg/kg.

The group of six rats (average weight 290 g) was administered a single ip dose of 100 mg/kg of n-butyronitrile solution [63]. The animals were observed until death or for 8 days; at that time the surviving rats were killed. Autopsies were performed on all rats, and n-butyronitrile and free and combined hydrogen cyanide concentrations were determined in the organs cited above. Two rats died within 24 hours of exposure, and four were killed after 8 days. In the two rats that died, average concentrations of n-butyronitrile in various organs were less than those found at previous doses. However, average concentrations of free and combined hydrogen cyanide were greater than those found at previous dose levels, especially in the heart, spleen, kidneys, stomach, and muscles. The animals that were killed after 8 days had only very small amounts or traces of

n-butyronitrile, as well as greatly reduced free and combined hydrogen cyanide concentrations, as compared with other doses.

Urinary excretion of n-butyronitrile and free and combined hydrogen cyanide also were measured over the 8-day period for the group of six rats [63]. n-Butyronitrile was still present in the urine through the 8th day. The authors believed that retention of n-butyronitrile was attenuated by pulmonary elimination, although it was still present in most of the organs after 8 days. In the third group, n-butyronitrile was found in the exhaled air, and 116 µg was found in the urine. The authors stated that pulmonary elimination was greater, although no data were given. Free and combined cyanides were eliminated in amounts greater than n-butyronitrile, especially during the first 2 days. The authors concluded that the slow urinary excretion and the relatively low solubility of n-butyronitrile in water were attributable to the number of carbon atoms in the aliphatic chain of the nitrile.

The lethal dose of n-butyronitrile for all the animals was 150 mg/kg [63]. As the size of the dose decreased, there was an increase in the time to death. There was a parallel increase in the ratios of the combined cyanides to the free cyanides with an increase in the time before death occurred. The authors concluded that the increase in the time before death allowed for more n-butyronitrile to be metabolized and transformed to free and combined cyanides. This was supported in the case of the second group with regard to the average concentrations of combined hydrogen cyanide, especially in the brain. What remains unclear, however, is which compound was responsible for toxic effects at the cellular level.

Szabo and Reynolds [62], in 1975, reported effects of n-butyronitrile on the duodenum and adrenal glands in rats. Female Sprague-Dawley rats (200 g) were given sc doses of n-butyronitrile three times a day at 100 mg/kg on days 1 and 2 and 200 mg/kg on days 3 and 4. The animals were autopsied soon after death or were killed and examined on the 5th day. Forty percent of the rats died as a result of the exposure. The authors reported that 80% of the rats had developed duodenal ulcers and 20% showed adrenocortical necrosis.

In 1971, Tsurumi and Kawada [64] reported on studies of the toxicity of ISOBUTYRONITRILE in animals. Acute toxicity studies were conducted using an ip route of administration in an unspecified number of male mice weighing 17-20 g each. Doses ranged from 0.4 to 0.8 g/10 g. Immediately following injection, the mice were considered to have signs of slight hyperkinesia. The frequency and amplitude of respiration also had increased. A few minutes after injection, they rolled to their sides. Clonic movements of the limbs were observed. The frequency of respiration decreased, and sensitivity to pain at an unspecified site diminished gradually and then disappeared. Corneal reflexes remained normal. Their respirations stopped altogether 20-30 minutes after injection, and

autopsies were performed. The hearts were in a state of general dilatation, no contraction of the ventricles was observed, but feeble contractions of the atria were still present. The authors concluded that, in mice, isobutyronitrile induced a central paralyzing effect and death by inhibition of respiration. The lethal dose of isobutyronitrile administered ip to mice was assumed to be below 38.6 mg/kg, but the exact lethal dose could not be determined due to the potency of the compound and the difficulty of administering a smaller dose.

Another study [64] of acute toxicity used an unspecified number of Wistar-strain female rats weighing 130-150 g. Apparently, a single dose of isobutyronitrile was administered either ip or orally at seven to eight different dose levels to groups of six animals. The LD_{50} values after 72 hours by both routes of administration were calculated. The signs observed in rats after ip or oral administration were similar to those observed in mice. The ip LD_{50} was calculated as 0.2 g/kg, and the oral LD_{50} was calculated as 0.98 ml/kg.

The acute toxicity of isobutyronitrile by inhalation also was studied in mice and rats [64]. Fifty milliliters of isobutyronitrile were placed in the bottom of an exposure chamber 18 cm in diameter, and the lid was closed. The isobutyronitrile vaporized naturally at a temperature of 20 C for 10 minutes, producing a chamber atmosphere nominally saturated with isobutyronitrile. Animals then were placed on a metal net above the liquid. Fifty mice and 50 rats of unspecified strain, sex, and weight were exposed in subgroups of 5 mice or 2 rats for various lengths of time. After exposure, the animals were returned to a normal environment and observed for 24 hours. The fraction of deaths occurring as a function of exposure time is shown in Table III-1. Signs similar to those described for ip and oral exposure were observed. The authors concluded that mice are more sensitive than rats to isobutyronitrile.

The effect of iv-administered isobutyronitrile on cardiac function was studied in 2.5-kg rabbits [64]. Information on age, sex, strain, or the number of animals used was not specified. At doses below 0.01 mg/kg, isobutyronitrile produced no remarkable effects. At doses higher than 0.01 mg/kg, there was a decrease in blood pressure, blood flow, and respiration. A further dose of 0.1 mg/kg resulted in death of all the animals. The authors observed a decrease in blood pressure, blood flow, and respiration rate and a decrease in the frequency of heartbeats. The heart ceased functioning after 30-40 minutes.

The authors [64] concluded that the direct cause of death after administration of isobutyronitrile was respiratory arrest from depression of the central mechanism of ventilatory activity. This conclusion was supported by recordings on the electrocardiogram (ECG) of continued heartbeats after respiration had ceased.

TABLE III-1

DEATHS WITHIN 24 HOURS OF EXPOSURE OF
MICE AND RATS TO ISOBUTYRONITRILE AT NOMINAL SATURATION IN AIR

Species	Exposure Time (minutes)	Deaths
Mice	2.0	10/10
"	1.5	7/10
"	1.0	5/10
"	0.5	3/10
"	0.25	0/10
Rats	10.0	10/10
"	8.0	6/10
"	6.0	4/10
"	5.0	1/10
"	4.0	0/10

Adapted from reference 64

Local effects of isobutyronitrile also were investigated in rabbits [64]. Isobutyronitrile was applied to the conjunctiva of one eye of each rabbit from a dropper and removed after 1 minute. Doses of 7.7 mg produced no remarkable effects, whereas doses of 15.5 mg produced reddening of the eyelids and conjunctiva, edema, and tearing. Increased doses of isobutyronitrile resulted in loss of the corneal reflex. Isobutyronitrile was injected sc into one ear of each rabbit with the opposite ear used as the control. Doses of 7.7 mg produced no remarkable abnormality, and doses of 15.5 mg produced reddening at the injection sites. Effects became more distinctive in proportion to increased doses of isobutyronitrile, and the injection site showed a whitened and damaged center surrounded by an erythematous periphery.

The effects of repeated exposure to isobutyronitrile were studied in blood and other tissues of 80 male and female Wistar rats, initially weighing approximately 160 g each [64]. The rats were divided into 8 groups of 10 animals of the same sex. For each sex, there were three exposure groups and one control group. Each exposure group received isobutyronitrile once daily for 14 days by one of the following doses and routes: 23.2 mg/kg or 38.6 mg/kg administered ip or 0.2 g/kg given orally.

No signs of toxic effects or deaths occurred during the course of administration [64]. Male rats receiving 0.2 g/kg orally had the lowest

mean body weights during the period of administration relative to the other male exposure groups and the male control group. From the data provided, this group also showed the least average increase in weight during the same period relative to each of the male groups. No significant changes in values for erythrocyte count, hematocrit, hemoglobin, specific gravity, or differential count were found in any of the exposed groups relative to their control group. Male and female groups receiving 0.2 g/kg orally did show lower leukocyte counts relative to their respective control groups. Male and female groups receiving either ip dose of isobutyronitrile did not show significant changes in leukocyte count relative to their controls. No significant changes were found in the serum enzyme studies--serum glutamic pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT), and alkaline phosphatase--for any of the groups. No significant differences among organ weights were found in animals receiving either ip dose of isobutyronitrile. Male and female groups receiving 0.2 g/kg orally showed slight weight increases of the stomach, liver, and adrenal glands relative to the respective control group. No significant differences were found for other organ weights for the oral exposure groups. No light microscopic changes were detected in the thymus, heart, lungs, stomach, spleen, kidneys, adrenal glands, and testes or ovaries in animals receiving 50 µl/kg of isobutyronitrile. The authors reported that rats exposed at 38.6 mg/kg showed definite parenchymatous degeneration of the liver and that male rats showed a greater degree of degeneration than females. Considering the liver cell degeneration and the earlier mentioned findings of increased organ weight of the liver in both sexes, the authors concluded that isobutyronitrile caused liver damage.

(b) Cyanohydrins

In 1960, Wolfsie [40] reported the results of animal studies using 0.05% W/W aqueous solution of anhydrous GLYCOLONITRILE. An oral LD_{50} of 10 mg/kg was determined based on a single feeding of the solution to an unspecified number of male albino mice. Death occurred within 2 hours after exposure. Signs of intoxication resembled "those of cyanide poisoning." A dermal LD_{50} was determined to be between 105 and 130 mg/kg, based on the exposure of an unspecified number of albino rabbits. Some surviving rabbits showed mild skin irritation. In three albino rabbits, a single application of 0.05 ml of a 50% solution (26 mg) of glycolonitrile to the conjunctiva produced an immediate moderate local irritation, followed by convulsions and coma 15-30 minutes later. Within 68 minutes, all three animals were dead.

Wolfsie [40] also described a study in which seven mice, seven rats, and seven guinea pigs of unspecified strain, sex, or age were exposed in an unspecified manner to glycolonitrile at an average vapor concentration of 27 ppm in air for 8 hours. Six of seven mice and two of seven rats died. The remaining mouse and four more rats died within the next 18 hours. Other signs of toxicity included lethargy and slight eye tearing. No guinea pigs died during the 18 hours after the exposure.

Wolfsie [40] next described a study in which glycolonitrile was administered in the diet to an unspecified number of albino rats for 13 weeks. Male rats ingested up to 62 mg/kg/day and the females ingested up to 92 mg/kg/day with no observed effects. Rat serum thiocyanate concentrations were related to the dose of glycolonitrile, but usually no serum cyanide was present. In a separate experiment, cyanide was observed to appear in the serum soon after the ip injection of an unspecified amount of glycolonitrile into rats and was accompanied by an increase in serum thiocyanate concentrations. Cyanide and thiocyanate concentrations were said to be proportional to the injected doses of glycolonitrile. The author concluded that the toxicity of glycolonitrile in animals was related to the release of cyanide, with detoxification occurring by rapid conversion to thiocyanate.

An experimental investigation of ACETONE CYANOHYDRIN toxicity, by unstated routes of administration, in four animal species was conducted by Shkodich [65], in 1966, in conjunction with research intended for use in determining a maximum permissible concentration of acetone cyanohydrin in water basins. The mice showed the highest sensitivity to acetone cyanohydrin with an LD_{50} value of 2.9 mg/kg. The LD_{50} value for albino rats was 13.3 mg/kg; for guinea pigs, 9 mg/kg; and for rabbits, 13.5 mg/kg.

The possibility of cumulative effects by acetone cyanohydrin was studied using 20 white mice and 20 albino rats [65]. Acetone cyanohydrin was administered by an unspecified route in daily doses equivalent to one-fifth of the LD_{50} for the respective species over 20 days. Neither death nor other evidence of cumulative effects was found in either species during the experiment.

A study of chronic effects of acetone cyanohydrin at daily doses of 0.00005, 0.0005, 0.005, and 1.33 mg/kg for 6 months was conducted in 44 albino rats and 16 rabbits [65]. The number and frequency of doses and route of administration were not specified. At the termination of the study, the animals were killed, and weight coefficients and vitamin C concentration of internal organs and the content of -SH groups in the gray matter of the cerebral cortex were determined. At doses of 1.33 mg/kg, rats exhibited the following effects at $P < 0.01$: an increase in erythrocytes, reticulocytes, and hemoglobin; an increase in vitamin C in the liver and adrenals; a decrease in the content of -SH groups in the brain; and decreases in the activities of serum catalase and cholinesterase. Also, at doses of 1.33 mg/kg, rabbits showed what was described as a disturbance in glycogenic function in the liver indicated by the slower utilization of galactose ($P < 0.05$) and a decrease in the content of -SH groups in blood serum.

At doses of 1.33 mg/kg and 0.0005 mg/kg, functional changes in higher nervous activity (attenuation of the processes of internal inhibition and a certain intensification of the excitatory process) were observed in rats

[65]. Also at doses of 0.0005 mg/kg, rats showed changes in the morphologic composition of the blood, catalase and cholinesterase activities, and vitamin C content, although no quantities were mentioned. Rabbits did not show noticeable effects in any of the tissues that were examined after doses of 0.005 mg/kg or 0.0005 mg/kg were administered. At doses of 0.00005 mg/kg, neither species showed any significant effects in the tissues or systems observed.

Motoc and associates [66] administered 5 mg of acetone cyanohydrin orally twice a week for 3, 5, or 8 months or 1 ml of acetone cyanohydrin in 84 liters (10.2 g/cu m) by inhalation twice a week for 3, 5, or 8 months to white rats in groups of 50 at each dose level. After exposure, the animals were killed. Blood samples were obtained for analysis of serum enzymes including leucinaminopeptidase, SGOT, SGPT, and glucose-6-phosphate dehydrogenase (G-6-PD). Concentrations of total proteins, electrophoretic fractions, and glycoproteins in the serum also were determined. Microscopic examinations of sections of the liver and kidney were conducted in all the animals. In addition, the stomachs of the rats exposed orally, and the lungs of those exposed by inhalation were examined microscopically.

Acetone cyanohydrin, apparently administered orally, produced a decrease of about 15% in the serum total proteins, a decrease in the albumin/globulin ratio, and an increase in gamma globulins [66]. Serum glycoproteins increased after 3 months of exposure. This was followed by a decrease below the mean, and, after 8 months of exposure, by a gradual increase without a return to the normal concentration. Beta-glucuronidase enzyme activity increased initially but dropped after prolonged exposure. Transaminase, aldolase, and leucinaminopeptidase activities were stated to have increased after initial exposures although no values were given. Changes in hepatic protein metabolism after increased duration of exposure were preceded by increases in leucinaminopeptidase, glutamic oxaloacetic transaminase, and glutamic pyruvic transaminase activities.

Acetone cyanohydrin administered orally produced various lesions in the stomach, liver, and kidney [66]. Stomach lesions ranged from increased gastric gland secretions to ulcerations, which became deeper and more extensive with increased duration of exposure. In the liver, both reversible and irreversible dystrophic changes were present. Reversible lesions became less frequent with increased exposure. The liver lesions were briefly described and illustrated. The pictured hepatic cell abnormalities included karyopyknosis, anisokaryosis, abnormal fat deposits, patchy thinning of cytoplasm, and absence of cytoplasmic granules. The livers of those animals exposed to acetone cyanohydrin by inhalation also had some degree of necrosis. Kidney lesions became predominantly irreversible during the longest period of exposure but were not as severe as those found in the liver.

Inhalation of acetone cyanohydrin produced lesions in the lungs with desquamation of bronchial epithelium, progressing to superficial

ulcerations associated with inflammatory infiltrates. Kidney lesions that encompassed the entire nephron and became irreversible during the longest period of exposure were also evident.

The authors [66] found that acetone cyanohydrin administered to white rats orally and by inhalation caused serious hepatic and kidney lesions that became irreversible with prolonged duration of exposure. The oral route of administration produced the most marked hepatic lesions and the most significant biochemical changes. An association was found between the presence and severity of tissue lesions and the degree of change in the serum concentrations of glycoproteins and albumin and the activities of leucinaminopeptidase, SGOT, SGPT, G-6-PD, aldolase, and beta-glucuronidase. Motoc et al suggested that these may be useful as indicators of toxic effects. Microscopic findings of regenerative zones and reversible lesions led them to conclude that adaptation mechanisms could possibly exist. Finally, the authors found that biochemical changes occurred sooner than histologic lesions, especially with regard to hepatic effects. Thus, these biochemical tests may be useful for monitoring subclinical changes induced by acetone cyanohydrin exposure and for detecting the potential for future pathologic changes.

Sunderman and Kincaid [2] studied the toxicity of acetone cyanohydrin in animals in 1953. Twenty-eight albino guinea pigs were studied for acute toxicity by skin absorption of a commercially prepared mixture consisting of 94.7% acetone cyanohydrin and 0.25% hydrogen cyanide; the authors thought the balance was water. The mixture was pipetted onto cheesecloth patches, 2.5-cm-square, which were applied to shaved abdomens of guinea pigs. The thickness of the patches varied according to the dose. At doses of 0.1 ml or less, one thickness of cheesecloth was applied; at doses of 0.2 ml or 0.5 ml, three thicknesses of cheesecloth were applied. The patches were secured with a 5-cm-wide band of adhesive tape around the animal. Observations were continued until either death or complete recovery occurred. The percutaneous LD_{50} was 0.14 g/kg.

Initial attempts by Sunderman and Kincaid [2] to study the toxicity of acetone cyanohydrin in rats by inhalation exposure led to the conclusion that contamination of vapor with free hydrogen cyanide affected the results. Reproducible results were obtained after 250 ml of commercially prepared acetone cyanohydrin, similar to that used for the skin exposure study, was placed in a saturator maintained at 23 ±1 C. Air from a drying tower was passed through the saturator into the chamber. The authors stated that the following results should be regarded as range-finding values: Mortality occurred in 50% of the rats within approximately 10 minutes after exposure to the saturated, purified acetone cyanohydrin vapor; 15 rats died after an average exposure time of 11 minutes; the rats collapsed within an average time of 4 minutes, but death occurred at a later time; recovery often occurred if the rats were removed from the chamber before cessation of respiration; when acetone cyanohydrin was

purified by the described method and allowed to remain at room temperature for 3 days, the toxicity of its vapor did not increase. Thus, the authors concluded that acetone cyanohydrin was the toxic agent in the vapor phase rather than newly formed hydrogen cyanide.

Sunderman and Kincaid [2] studied the effectiveness of treating acetone cyanohydrin poisoning by an adaptation of the procedure described by Chen et al [67], in 1944, for treatment of cyanide poisoning. Groups of five rats were exposed to acetone cyanohydrin vapor until respiration stopped in one of the five rats. Of the remaining four in each group, two were kept as controls and two were given treatment. Amyl nitrite, sodium nitrite, or sodium thiosulfate was administered at doses calculated to be equivalent to those recommended for administration of these cyanide antidotes to humans on the basis of relative body weights. In the control groups, all 16 untreated rats died within 1 minute after exposure ended. Amyl nitrite, administered after exposure to acetone cyanohydrin vapor, increased the survival time of four rats by 10 or more minutes, but all except one died. When sodium nitrite and sodium thiosulfate were administered separately to a pair of rats after exposure, one of each pair survived. When a combination of sodium nitrite and sodium thiosulfate was administered to two rats after exposure, both survived. The administration of sodium nitrite and sodium thiosulfate separately or in combination prior to exposure resulted in no deaths in each group of two rats so treated.

The authors [2] concluded that nitrites and sodium thiosulfate were effective for treating acetone cyanohydrin vapor poisoning in rats. Further, the effectiveness of this mode of treatment supported the view that the toxic action of acetone cyanohydrin was associated with the in vivo release of hydrogen cyanide as acetone cyanohydrin dissociated into acetone and hydrogen cyanide. The authors inferred that the mode of treatment described by Chen et al [67] relative to hydrogen cyanide poisoning in humans should also be effective for treating acetone cyanohydrin poisoning in humans because its toxic effects were mediated by the release of cyanide.

Krefft [41], in 1955, compared the effects of skin exposure to acetone cyanohydrin and aqueous potassium cyanide solution in guinea pigs. Three male guinea pigs were dermally exposed to acetone cyanohydrin, and one female was dermally exposed to 10% aqueous potassium cyanide solution. Three test animals were each exposed to about 1.5-2 cc of acetone cyanohydrin either by painting both ears, painting a small area of the shaved back, or by application of a 2- x 5-cm gauze sponge to the shaved area of skin on the back. All three were restless initially; pulse and respiration rates increased within 1 minute; within 7 minutes, all the animals showed difficulty in breathing, and there was excretion of urine and feces; respiratory paralysis and snapping movements of the snout occurred within 31-40 minutes; death occurred in all three animals within 48-51 minutes. Restlessness and a slight increase in the respiration rate

were also observed in one male animal exposed to about 10 cc of acetone by application of a 2- x 4-cm gauze sponge to the shaved back.

The single guinea pig exposed to potassium cyanide solution by a 3- x 4-cm gauze sponge applied on the shaved skin of the back showed similar effects as those previously described for the animals exposed to acetone cyanohydrin [41]. Spasms attributed to anoxia were observed within 34 minutes, and death occurred within 60 minutes.

Autopsy on animals exposed to acetone cyanohydrin and potassium cyanide showed local hyperemia and swelling of exposed areas, lung edema, bright red blood, hyperemia and edema of the liver and the brain, severe dilatation of the right side of the heart, extensive subpleural and subepicardial echymoses, and acute venous blood obstruction in the major organs and exposed areas of the skin [41]. A distinct bitter almond odor was present in the viscera. The author indicated that the intoxication phenomena were the same with respect to acetone cyanohydrin and potassium cyanide solution, except that acetone cyanohydrin was apparently absorbed faster because of its lipid solubility. The clinical signs resembled those of delayed hydrocyanic acid intoxication. In addition, the author commented that these animal tests show extensive parallels to human cases of death as a result of acetone cyanohydrin exposure.

(c) Dinitriles

In 1969, Panov [68] reported the effects of MALONONITRILE administered by various routes in mice, rats, and rabbits. In the first experiment, five mice (18-20 g) were exposed to vapor of malononitrile for 2 hours in an exposure chamber maintained at 18-20 C. The concentration of malononitrile vapor was not stated, but it was implied that the air in the chamber had been allowed to come into equilibrium with molten malononitrile. Six additional mice (18-20 g) were exposed for 2 hours in an exposure chamber maintained at 29-30 C, at concentrations of malononitrile ranging from 8 to 300 mg/cu m of air. The actual concentrations of malononitrile during these dynamic trials were checked colorimetrically twice during each experiment. In a second study, a total of 100 white mice (18-20 g) and an unspecified number of rats (260-270 g) were administered oral doses of 5-50 mg/kg of malononitrile. In a third experiment, molten malononitrile was applied for 2 hours to the tail skin of healthy white mice and applied on the shaved thigh of four chinchilla rabbits. The skin was washed 1 hour later. Two to three drops of molten malononitrile were applied in the conjunctival sac of six additional rabbits. The animals were observed for 14 days and then killed. All animals were examined for morphologic changes in selected organs.

In the inhalation study, Panov [68] reported that the white mice receiving a single exposure of malononitrile developed signs of restlessness and an increse in the respiration rate in the early

posttreatment period followed by lassitude, decrease in respiration rate, cyanosis, incoordination of movements, trembling, convulsions, and, in some animals, eventual death. None of the five animals in the chamber maintained at 18-20 C died; however, two of six mice in the chamber maintained at 29-30 C died. Fifty percent of white mice and rats exposed at 200-300 mg/cu m of malononitrile died; furthermore, at a concentration of 240 mg/cu m, the mice developed hyperemia and had increased weight coefficients of the lungs (10.0 ±0.39), kidney (21.0 ±0.90), and brain (19.7 ±0.7), compared with those of the control group with weight coefficients of 7.8 ±0.4, 16.6 ±0.76, and 16.5 ±0.77, respectively. The liver showed a decrease in weight coefficient (21.0 ±0.90) in those treated, compared with that in the controls (60.2 ±3.2).

After single oral administrations of 5-50 mg/kg of malononitrile, general intoxication was observed [68]. At 5 mg/kg, no mice died; however, at doses of 20-30 mg/kg, 60-80% of the mice died, whereas a 100% mortality occurred in those animals administered doses of malononitrile ranging from 40 to 50 mg/kg body weight. Further, at higher doses, a moderate destruction of the mucosa of the stomach, a general hyperemia of all organs, and "dystrophy" of intracellular fats and proteins accompanied by leukocytic infiltration of the gastric mucosa were observed. At lower doses of 5-20 mg/kg, no morphologic changes were observed in the mice that survived the exposure period. The LD_{50} was 18.6 mg/kg for mice and about 25 mg/kg for rats.

Panov [68] reported that direct application of warm malononitrile produced the following effects in the eyes of all six rabbits: tearing, hyperemia of the conjunctiva, and spasm and swelling of the eyelids. Respiratory impairment, convulsions, and death occurred in four of the six rabbits.

After the tails of the mice were wet by malononitrile, the animals showed signs of restlessness, rapid respiration, and slight cyanosis of the mucosa of the lips and extremities [68]. The symptoms subsided following removal of the chemical by washing. Panov also observed trembling and skin redness following exposure of the skin of the thigh of the rabbit to malononitrile.

Panov [69], in 1970, reported the effects of exposing a group of 10 rats with an initial mean body weight of 276.5 g to malononitrile vapor. The exposures were at a concentration of 36 mg/cu m for 2 hours/day for 35 days in a dynamic chamber maintained at a temperature of 29-30 C. The malononitrile was analyzed at 2-day intervals by determining the amount of nitrogen with Nessler reagent. Ten additional rats were used as controls. Following exposures, the treated and control rats were examined for changes in body temperature, weight, blood counts, and hemoglobin levels. Selected organs also were removed and weighed at autopsy 7 days after the end of exposure. The author measured the consumption of pure oxygen before and

after carbon dioxide inhalation and the consumption of oxygen by homogenized lung tissue, as determined with the Warburg apparatus. The effects of malononitrile on the CNS were measured by "the index of summation threshold." Also, the author examined the effects of malononitrile on hepatic function by measuring the amount of hippuric acid excreted in a 24-hour period following ingestion of 400 mg/kg of sodium benzoate.

Panov [69] found that the body weight of the experimental rats did not differ significantly from those of the controls. However, the ratio of lung weight to body weight increased in the treated rats, compared with that in the controls. There was a minor change in body temperature from days 19 through 35 in the treated rats. On day 35, the temperature was 36.8 C in the treated rats, as compared with one of 35 C in the control rats. A slight decrease in the concentration in blood of hemoglobin and an increase in that of reticulocytes were also observed. The reticulocyte count was 34 in the treated rats and 14.1 in the controls on the 7th day of treatment. On the 35th day, the reticulocyte count was 27.9 in the treated group and 10.3 in the controls. A decrease in the amount of hippuric acid in 24-hour urine samples of the treated animals was observed. However, there was a concomitant decrease in volume of urine excreted; therefore, the author reported no significant difference in hippuric acid excreted per ml of urine. The author also noted no significant difference in the "index of summation threshold" of the CNS in treated and control rats. Furthermore, the rate of oxygen consumption did not vary significantly in the experimental animals, as compared with that in the controls. Panov concluded that 36 mg/cu m of malononitrile was slightly toxic to rats; this toxicity was manifested mostly by its effect on red blood cells, viz, the hemoglobin level was down and the reticulocyte count was elevated.

Van Breemen and Hiraoka [70], in 1961, described, in an abstract, preliminary investigations on the effects of malononitrile on the cells of the spinal ganglia of rats. Rats were administered, through unspecified routes, 6 to 8 mg/kg of malononitrile. The authors reported the following nuclear and cytoplasmic changes in nerve and satellite cells of spinal ganglia: (1) an increase in the size of the nuclear pore, (2) an increase in the breakdown of endoplasmic reticulum into microvesicular units and an increase in the amount of dense material within the endoplasmic reticulum, (3) an increase in number and size of the Golgi vesicles, (4) an increase in the cytoplasmic pigment granules in the bodies of the nerve cells of the spinal ganglia, and (5) an increase in dense cytoplasmic granules in the satellite cells.

Rats administered (route not specified) 1.2 mg/kg malononitrile for 8 days developed cytoplasmic changes in the neurons [70]. This was demonstrated by the presence of vacuoles containing short filamentous structures in the cytoplasm along the periphery of the cell body and the

development of open spaces between Nissl bodies, which gave the appearance of increased fluidity of the neuronal cytoplasm in the treated animals.

Hicks [71], in 1950, reported the effects of malononitrile on the brain and other tissues in rats. Twenty-six young adult rats were administered ip doses of 5-10 mg/kg of malononitrile at 2- to 4-hour intervals for 1-2 days. The animals were killed, and complete autopsies were performed. All major organs were prepared for examination by conventional histologic methods.

Hicks [71] reported that malononitrile induced brain lesions in rats. Four rats died during the acute phase of malononitrile treatment. Fifteen of the 22 rats that survived had brain lesions, but the other 7 survivors had no discernible damage. The results of the autopsies of four representative rats showed brain lesions in the corpus striatum involving both the gray and white matter. In "one or two" of these rats, necrosis occurred in the striatal neurons, with accompanying proliferation of microglia and oligodendroglia, 1-2 days following treatment. Some striatal neurons of the white matter showed no reaction. The author also observed demyelinating lesions of the optic tract and nerve, lesions of the cerebral cortex, involving the rhinal fissure and cortical areas 51a and 51b, and lesions of the olfactory bulb and substantia nigra.

Hicks [71] also reported visceral changes, such as ventricular myocardial changes, in most of the experimental rats. Moderate patchy renal tubular necrosis was evident, and a few animals that died during the acute phase of treatment had developed pulmonary edema. An unstated number of rats showed elongation and vacuolation of the thyroid acinar cells and mitotic figures in the parathyroid cells.

The author [71] concluded that to some extent malononitrile exerts tissue specificity in its action. He could not precisely explain the nature of the effects observed, but he postulated that the differential tissue susceptibility was due to varying factors: duration of action of the injected chemical, rate of detoxification and excretion, selective permeability of tissues, qualitative differences in tissue metabolism, and inhibition of cellular respiration due to a reaction of released cyanide with cytochrome oxidase.

Stern et al [72], in 1952, reported on two studies on the metabolism of malononitrile by rat brain, liver, and kidney tissue in vitro. In the first study, malononitrile at a concentration of 0.01 M was added to three incubation media (Krebs III, modified Krebs III, and bicarbonate) containing tissue slices of brain, liver, and kidney. Respiration and glycolysis were then determined. In addition, cozymase levels were determined by the apozymase test. Respiration was measured manometrically. In the second study, respiration and anaerobic glycolysis were simultaneously determined in the rat and guinea pig tissue by Warburg's

two-vessel method. Anaerobic glycolysis was determined manometrically for guinea pig brain slices. The tissue slices were incubated at 37 C for 2 hours.

In an additional experiment [72], homogenates and extracts of brain, liver, and kidney tissue were analyzed to determine thiocyanate formation in rat liver extract, effects of tissue homogenates and extracts on malononitrile in the presence or absence of thiosulfate, and the effect of malononitrile on rhodanase activity.

Stern and his associates [72] found that respiration of brain, kidney, and liver slices was inhibited by 0.01 M malononitrile. In a bicarbonate medium, brain and kidney respiration were not markedly inhibited by 0.01 M malononitrile during the 1st hour but decreased during the 2nd hour for both the rat and the guinea pig. Also, the 2nd hour anaerobic glycolysis in guinea pig brain tissue decreased to approximately 50% of the 1st-hour value. Thiosulfate did not prevent the inhibition of anaerobic glycolysis produced by malononitrile. Malononitrile increased the lactic acid content of rat brain slices within 1-2 hours but had no effect on the NAD concentration. The formation of thiocyanate from malononitrile and thiosulfate was highest in the presence of liver tissue, lowest with brain, and intermediate with kidney.

The enzyme rhodanase, which catalyzed the formation of thiocyanate from cyanide and thiosulfate, was ineffective for catalysis of thiocyanate formation from malononitrile. The observed thiocyanate formation in vivo, therefore, apparently came from an intermediate metabolite and not the parent malononitrile molecule itself. Malononitrile inhibited respiration and increased the relative ratio of aerobic to anaerobic glycolysis. The authors did not postulate a mechanism for the effect of malononitrile on cellular respiration.

In 1952, Macht [73] reported the effects of single or repeated injections of SUCCINONITRILE in mice, rats, guinea pigs, rabbits, and cats. In the first series of experiments, the author investigated the toxicity and mean lethal dose. An unspecified number of rats and cats, 200 mice, 10 rabbits, and 30 guinea pigs were administered single ip doses of 5% succinonitrile. Eight rabbits were administered iv or im doses of 50 mg of succinonitrile five times/week for 3 weeks. The author did not report the weights of the animals. Mean lethal doses of 50 mg/kg, 250 mg/kg, "60 and 50 mg/kg," 23 mg/kg, and 80 mg/kg were determined for mice, rats, guinea pigs, rabbits, and cats, respectively. In the mice, rats, and guinea pigs, convulsions and signs of asphyxia developed after administration of mean lethal doses of succinonitrile by various routes. No toxic effects on the neuromuscular apparatus were observed in rabbits. Macht concluded that the toxicity of succinonitrile was low in mice, rats, guinea pigs, and rabbits. No control animals were used, although their use would have been appropriate in the chronic study of rabbits.

The second series of experiments was conducted to determine the effects of succinonitrile on blood pressure, respiration, and hepatic and renal function [73]. An unspecified number of rabbits and cats were anesthetized with pentobarbital and administered 1 ml of succinonitrile iv. No effects on the blood pressure and heart rate were observed in the rabbits and cats, and no impairment of kidney or hepatic function was observed in four of the rabbits. A transient increase in the frequency of respiration was observed after administration of iv doses of 5% succinonitrile. Although the author reported no apparent toxic effects in the gastrointestinal tract and neuromuscular systems, some animals developed diarrhea after repeated injections of succinonitrile.

Contessa and Santi [74], in 1973, investigated the release of cyanide from succinonitrile in rats and rabbits in vivo and in vitro. In the in vivo studies, male rabbits weighing 2-3 kg and male rats weighing 400-450 g were administered succinonitrile in iv doses of 25-40 mg/kg and 25-50 mg/kg, respectively. Levels of cyanide and thiocyanate were determined in samples of urine from rabbits and rats. The total number of rats that were studied was not mentioned. In the in vitro studies, levels of cyanide and thiocyanate were determined in liver slices and homogenates of rat or rabbit liver or with isolated mitochondrial, microsomal, and soluble fractions. Samples (0.1-2.0 ml) of filtered 24-hour urine output were collected from groups of two rats and from each rabbit pre- and postadministration with succinonitrile to determine thiocyanate concentrations. In another study, rats were pretreated with 2 ml/kg of carbon tetrachloride sc 48 hours prior to succinonitrile administration. Filtered urine samples were collected, and cyanide concentrations were determined colorimetrically.

Contessa and Santi [74] reported a sixfold increase in urinary thiocyanate following iv-injected doses of 25 mg/kg of succinonitrile compared with the controls. Forty-eight to 120 hours postadministration of succinonitrile, thiocyanate levels approached control values. Pretreatment of rats by sc injection of 2 ml/kg carbon tetrachloride inhibited urinary thiocyanate excretion. Urinary excretion of cyanide increased over fivefold 48 hours posttreatment with succinonitrile. The values at 72-120 hours posttreatment approached normal values.

The authors also reported that rat and rabbit liver slices catalyzed the release of cyanide from succinonitrile [74]. However, 0.1-2.0 mg/g of Triton-X-100 strongly inhibited cyanide release from succinonitrile in rat liver slices. Liver slices of rats pretreated with carbon tetrachloride did not release detectable amounts of cyanide from succinonitrile.

Contessa and Santi [74] concluded that rabbits and rats converted about 60% of the administered succinonitrile to cyanide, which was excreted as thiocyanate. The release of cyanide was inhibited by carbon tetrachloride and Triton-X-100. The authors also postulated that disruption of the liver

cells, as by centrifugation to separate their contents, depressed or eliminated their ability to liberate cyanide ions or to form thiocyanate. The authors proposed that cellular membranes contain enzymes or enzyme complexes that are responsible for the conversion of succinonitrile to cyanide, which is excreted as thiocyanate. However, following homogenization, these enzyme or enzyme complexes may be destroyed or damaged by homogenization.

In 1972, Cavanna and Pocchiari [75] investigated the fate of ^{14}C-labeled succinonitrile in male mice. They reported that a mean of 53% of the total succinonitrile injected ip in each animal in single- and multiple-dose studies was eliminated in the first 24 hours posttreatment and 88% of this eliminated succinonitrile was excreted as metabolites. In a single-dose study, 7% of the ^{14}C-labeled succinonitrile was excreted as thiocyanate and 36% as intermediate metabolites in 24 hours. In the multiple-dose study, 18% thiocyanate and 24% intermediate metabolites were excreted.

Cavanna and Pocchiari [75] concluded that succinonitrile was either excreted unmetabolized or was metabolized to cyanide and excreted as thiocyanate in mice within 24 hours posttreatment. The authors postulated that the high percentage of metabolites excreted in 24-hour urine samples may be due to the presence of two reactive centers in the succinonitrile molecule, resulting in the formation of two intermediate metabolites, diethylene cyanohydrin and cyanoacetic acid.

In 1975, Curry [76] reported on his investigation of the excretion of succinonitrile and its metabolites in urine and feces of mice, following a single injection of succinonitrile. The cumulative excretion of succinonitrile and metabolites in urine and feces measured 60% by 24 hours and 83% by 72 hours. In the first multiple-dose experiment in which mice received three doses of unlabeled succinonitrile and one dose of radioactive succinonitrile, 52% of the radioactivity was excreted in urine in 24 hours. In the second multiple-dose experiment, in which mice received four doses of radioactive succinonitrile, 50% of the radioactivity was excreted in each 24-hour period.

Curry [76] concluded that a fraction of the succinonitrile was excreted unmetabolized or converted to cyanide and excreted as thiocyanate in the 24 hours following its administration. After 24 hours, virtually all the metabolites were excreted. The highest rate for thiocyanate excretion occurred 2-6 hours after administration. After 48 hours, most of the excreted material was not extractable from water with chloroform or amyl alcohol, which suggested that highly polar, perhaps ionizable metabolites were formed. Curry speculated that one of the metabolites was cyanoacetic acid, a compound known to be derived from succinonitrile in rats. He further suggested that metabolic hydroxylation of the methylene group would form an unstable cyanohydrin that in turn would release cyanide. The

release of cyanide from succinonitrile in vivo and in vitro [74-76] further supports the possibility that toxicity of nitriles may be due to release of a cyanide radical. When alpha-hydroxylation occurs, the resulting cyanohydrin can readily dissociate under biologic conditions to release C≡N ions.

Harger and Hulpieu [77], in a 1949 abstract, described the effects of TETRAMETHYLSUCCINONITRILE in rats, guinea pigs, rabbits, and dogs and the influence of thiosulfate, nitrite, and barbiturates on the toxicity of tetramethylsuccinonitrile. Animals treated with tetramethylsuccinonitrile developed violent convulsions and asphyxia, which eventually led to death of the animals from 1 minute to 5 hours following the convulsions. Oral administration of tetramethylsuccinonitrile at 49 and 56 mg/kg induced convulsions in rats after 5 hours, and they died several hours later. Rats that inhaled vapor of the chemical at 60 ppm for 2-3 hours died. At a lower concentration, 6 ppm for 30 hours, the animals also died.

Tetramethylsuccinonitrile LD_{50} values were determined in three animal species: 30 and 23 mg/kg for rats and guinea pigs, respectively, after sc administration [77], and 20 mg/kg for rabbits after iv injection. A dose of 20 mg/kg given sc was lethal to dogs. The authors reported no influence of unspecified doses of sodium thiosulfate and sodium nitrite on the toxicity of tetramethylsuccinonitrile; however, administration of a quick-acting barbiturate (identity of the compound and route not specified) followed by phenobarbital did reduce the toxicity of tetramethyl-succinonitrile given in doses up to 50 mg/kg. Sodium thiosulfate and sodium nitrite have been reported to be effective antidotes following other nitrile poisonings. The failure of the authors to observe a reduction in the toxicity of tetramethylsuccinonitrile may well have been due to the administration of an inadequate dose of the antidotes or to the failure to administer the antidote soon enough following exposure.

Svirbely and Floyd [78], in 1964, reported the results of a toxicologic study of ADIPONITRILE. Mongrel bitches fed "the equivalent of" 10, 100, 500, and 1,000 ppm of adiponitrile daily were tested for blood and urine abnormalities and for liver and kidney functions. Normal values were found for animals fed adiponitrile at 500 ppm or less, but those given 1,000 ppm daily were unable to consume the entire dose; the dogs either vomited the adiponitrile-containing food or failed to eat portions of it during the 1st week. Monitoring the thiocyanate excretion in the urine showed that the levels increased as the concentration of the ingested nitriles increased. Recovery of adiponitrile in the form of urinary thiocyanate averaged about 50%. Slight increases in the concentration of thiocyanate in the blood were found, and negligible amounts were found in feces.

In a 2-year study, male and female Carworth Farms-Wistar rats were given 0.5, 5.0, and 50 ppm of adiponitrile in their drinking water [78]. Daily water consumption was not affected by the different concentrations of

adiponitrile. No abnormalities were found after periodic hematologic studies with these rats. No appreciable differences in body weight were noted during the 2-year study. Advanced adrenal degeneration was found in female rats exposed at all three concentrations of adiponitrile and in males exposed at 50 ppm. Degeneration of other organs was noted but was attributed to aging of the rats. The ratios of organ weights (spleen, liver, and kidney) to total body weight at the conclusion of the study were not significant. Pregnant Sprague-Dawley rats were exposed to adiponitrile at 10, 100, and 500 ppm. Data reported from the first generation (two litters) did not indicate any decrease in fertility, gestation, or viability.

Ghiringhelli [16], in 1955, reported on the metabolism and toxicity of adiponitrile in guinea pigs and the effects of anticyanide treatment. Twenty guinea pigs, 4-8 months old and weighing 560-580 g, were administered sc unspecified quantities of a 5% aqueous solution of adiponitrile. In addition to observing the animals for toxic effects, blood hydrocyanic acid and urine (24-hour samples) thiocyanate levels were determined 1-3 days after injection. In a second study, 5 of 13 animals that survived adiponitrile administration were given a 25% solution of sodium thiosulfate at a dose of 2.5 ml/kg. Also, an unspecified number of guinea pigs were administered sodium nitrite sc at a dose of 70 mg/kg.

The author [16] reported that adiponitrile was toxic to guinea pigs. The lethal dose was estimated to be 50 mg/kg on the basis of 20 animals. Hydrogen cyanide concentrations in blood from the heart ranged from 0.12 to 1 mg% (mean of 0.68 mg%). The mean thiocyanate concentrations for the 24-hour urine samples on days 1, 2, and 3 following adiponitrile treatment were 4.6 mg/100 g, 3.5 mg/100 g, and 2.64 mg/100 g, respectively. The mean concentration of thiocyanate excreted in 24-hour urine samples from animals treated with adiponitrile and sodium thiosulfate were 5.23, 3.13, and 2.44 mg/100 g, respectively. Ghiringhelli concluded that adiponitrile was metabolized to hydrocyanic acid and excreted in urine as thiocyanate. He also stated that sodium thiosulfate was an effective antidote against adiponitrile poisoning, based on 92.8% survival of animals poisoned with a mean lethal dose of adiponitrile.

Correlation of Exposure and Effect

Humans absorb nitriles through the skin [2,10,40,42] and respiratory tract [10,31-33,35,37,38]. After absorption, nitriles may be metabolized to an alpha cyanohydrin or to inorganic cyanide, which is oxidized to thiocyanate and excreted in the urine. Nitriles also undergo other types of reactions, depending in part on the constitution of the moiety to which the C≡N group is attached. The C≡N group may be converted to a carboxylic acid derivative and ammonia or may be incorporated into cyanocobalamine.

Ionic cyanide reacts also with carboxyl groups and with disulfides. Nitriles and their metabolic products have been detected in urine, blood, and tissues [38].

Studies on the effects of the selected nitriles on humans, following exposure by various routes of administration, have revealed a variety of effects. These include constriction and numbness in the throat, increased salivation, nausea, anxiety, confusion, vertigo, giddiness, hyperpnea, labored breathing, and slow and irregular respiration. In some cases, palpitations, rapid, weak, and irregular pulse, coma, convulsions, trismus, and profuse sweating may occur. Depending on the extent of exposure, death by respiratory arrest may ensue [10,31,32]. These effects are similar to those associated with cyanide poisoning [86,87]. These observations of a close similarity to an underlying cyanide effect for acute poisoning by nitriles are further supported by the effectiveness of anticyanide therapy, sodium nitrite followed by sodium thiosulfate [67], in treatment of nitrile poisoning. There are no chronic effects associated with exposure of humans to cyanide [88]; but there is evidence of ulcerogenic effects in animals produced by sc injection of propionitrile and n-butyronitrile in rats [56,62], thyroid hyperemia and hyperplasia produced by sc injection of acetonitrile in rats [48,49], and CNS effects from therapeutic use of malononitrile and succinonitrile [26,27,30].

The effects produced by exposure to cyanide from a variety of sources, including nitriles, are respiratory difficulties, headache, muscular incoordination, varying degrees of mental confusion (progressing to deep coma), and either cyanosis or bright red color of the blood. Depending on the extent of poisoning, there may be convulsions of an anoxic nature, with involuntary urination and defecation. The circulation may be strong or weak, and the pupils are dilated. Vomiting frequently occurs before loss of consciousness, and the vomitus may have the odor of bitter almonds characteristic of cyanide. Pneumonia is a common sequela in nonfatal cases. In fatal cases of poisoning, death usually occurs during the first 30 minutes. Pathologic findings are those of asphyxia. The most affected tissues (stomach, mouth, and lungs) are often reddened. All portions of the CNS, including the corpus callosum and the substantia nigra, may show degenerative changes [71,87] (see Table III-2).

Although all the selected nitriles appear to have in common the release of cyanide for producing toxic effects, at least on an acute basis, there are substantial differences among nitriles in the amounts necessary to cause poisoning, in the durations of exposure, and in the time intervals between exposure and manifestation of these effects. These differences among the effects of the various nitriles may be partly related to the differences in the rate and extent of cyanide ion release.

The only mononitriles for which human exposure data are available [10,31,32,39] are acetonitrile and isobutyronitrile. The onset of illness

from an acute exposure of humans to isobutyronitrile appears to occur more rapidly [10,39] than that occurring from similar exposures to acetonitrile [31,32]. This may be explained by Amdur's suggestion [32] that the delayed effect of acetonitrile when compared with other members of the homologous series may be due, in part, to the more rapid rate of oxidation in vivo of the alkyl group of the higher homologs. The signs and symptoms of overexposure to these mononitriles include headache, dizziness, profuse sweating, vomiting, loss of consciousness, difficulty in breathing, and dilated pupils. In cases of severe exposure, coma followed by death has occurred [31,32]. Treatment for the acute effects includes use of sodium nitrite and sodium thiosulfate, the conventional therapy for cyanide poisoning.

The cyanohydrins can be absorbed through the skin or inhaled without the exposed individual being aware of exposure. The alpha-cyanohydrins will apparently dissociate readily to yield hydrogen cyanide and a ketone or aldehyde [2]. There is a delayed onset of illness, generally attributed to the time required for dissociation to produce free hydrogen cyanide. One investigation [39] suggested that the effects of exposure of humans to acetone cyanohydrin are similar to those produced by exposure to isobutyronitrile, a mononitrile. However, the amount of cyanohydrin and the time required for it to produce a toxic effect in humans appear to be less than those for exposure to a mononitrile.

A few cc of adiponitrile produced severe signs and symptoms in an 18-year-old man, requiring hospitalization [16]. Malononitrile has been used therapeutically at 2-4 mg/kg doses [26,27,29] for the treatment of certain mental disorders. Facial redness, tachycardia, and congestive flow of blood to the head were consistently observed during treatments [26]. Although the evidence is inconclusive, exposure to tetramethylsuccinonitrile at a low concentration in air may cause convulsions and loss of consciousness [43].

In the workplace, acute poisoning and death have been reported following inhalation of nitriles. Dequidt et al [33] reported a fatality in a man exposed to acetonitrile vapor (concentration and duration not known). Approximately 4 hours after leaving work, the worker experienced gastric and respiratory distress, vomiting, profuse perspiration, and coma. Death occurred 6 days after the onset of poisoning despite anticyanide therapy. Unmetabolized acetonitrile, free hydrogen cyanide, and combined cyanide were detected in the viscera, blood, and urine. Zeller et al [10] reported similar findings following a 10-minute exposure of a worker to isobutyronitrile vapor at an unknown concentration. However, the worker recovered following repeated doses of thiosulfate. Grabois [31] and Amdur [32] described the effects of exposure of approximately 15-20 workers to volatilized acetonitrile. One death and eight cases of intoxication were reported. Signs and symptoms following exposure were similar to those reported by Dequidt et al [33] and Zeller et al [10]. However, eight

survivors developed additional signs and symptoms including hypothermia, hypotension, and oliguria.

Dermal exposures to various nitriles have caused adverse reactions including death in some instances. Zeller et al [10] reported seven cases of minor skin irritation and inflammation developing about 5-15 minutes following exposure to adiponitrile. One of seven workers had serious skin destruction and required hospitalization for 117 days. In another incident [39], a worker had direct skin contact with acetone cyanohydrin (quantity unknown) for an unknown period of time while filling drums. He vomited within 5 minutes and became unconscious within 10 minutes. In these cases of skin contact [10,39], exposures also may have been by inhalation.

Wolfsie [40] reported two cases of skin exposure to unknown quantities of 70% aqueous solutions of glycolonitrile in an occupational setting. In addition to the characteristic signs and symptoms of cyanide poisoning, the author reported that there was incoherent speech. Sunderman and Kincaid [2] reported three cases of poisoning following direct skin contact with acetone cyanohydrin. One of the workers became nauseated, subsequently developed convulsions, and died 6.5 hours postexposure. Signs and symptoms observed in survivors were headache, nausea, vomiting, and cardiac palpitation. Lang and Stintzy [42] reported poisoning with acetone cyanohydrin following direct skin contact with the liquid for 40-60 minutes and with the dry residue for about 5.5 hours. Five hours after exposure, typical signs and symptoms of cyanide poisoning, including muscular spasm and a painful constriction of the throat, were observed.

Nitrile intoxication has been reported following ingestion in the workplace. Ghiringhelli [16] reported the poisoning of a worker who ingested a small amount of adiponitrile. Signs and symptoms were similar to those caused by nitrile intoxication dermally and by inhalation and included headache, dizziness, mental confusion, and loss of consciousness. In addition, both pupils were extremely dilated.

Only one report of long-term, low-level effects of occupational exposure to a nitrile has been found. Reinl [43] reported a study of 16 workers allegedly suffering ill effects from exposure to tetramethylsuccinonitrile vapor at an unknown concentration. The subjects complained of headache, dizziness, nausea, vomiting, a peculiar taste in the mouth, formation of excessive and frothy spittle, respiratory distress, insomnia, lapses of consciousness, and convulsions. The results of physical examination and limited laboratory tests of liver function and serum proteins revealed no consistent or characteristic abnormality. Two of the subjects examined had lost consciousness following acute exposures. The presence of tetramethylsuccinonitrile had not been proven in the work environment. However, the author conjectured that it was released as a thermal decomposition product of azo-isobutyronitrile, which was employed in the workplace as a polyvinyl chloride foaming agent.

One report, by Pozzani and associates [35], of experimental exposures of three men indicated the possibility of minor and transient effects from inhaling acetonitrile at relatively low concentrations for 4-hour periods. One of three volunteers exposed at 40 ppm described a slight tightness in the chest, which he characterized as a "cooling sensation" like that of menthol in the lungs. He also experienced a slight transitory flushing of the face 2 hours after inhalation and a slight feeling of bronchial tightness after 5 hours. No significant levels of cyanide were detected in the blood or urine of the subjects. All other volunteers exposed to acetonitrile at 40 or 80 ppm intermittently for up to 2 weeks showed no subjective responses. However, at 160 ppm one of two exposed volunteers had a slight flushing of the face, followed 5 hours later by a feeling of bronchial tightness.

Animal studies have been conducted to determine the effects of exposure to various nitriles by inhalation and dermal absorption. Inhalation studies with rats, monkeys, guinea pigs, and dogs consisted of exposing them to acetonitrile, propionitrile, n-butyronitrile, isobutyronitrile, glycolonitrile, acetone cyanohydrin, tetramethylsuccinonitrile, and malononitrile. These studies were at concentrations ranging from 6 to 53,000 ppm (Table III-3).

Pozzani et al [35] reported that rats exposed to acetonitrile at 53,000 ppm for 30 minutes died but survived at the same concentration when exposed for 15 minutes. Haguenoer and colleagues [55] reported that death of rats occurred 30 minutes after exposure to acetonitrile at 25,000 ppm. In dogs, death occurred within 14 hours from 4-hour exposures at 16,000 ppm [35]. In the same study, after 4-hour exposures, the LC_{50} values for rabbits and guinea pigs were 2,828 ppm and 5,655 ppm, respectively. Pozzani et al also reported hyperexcitability, incoordination, and subdural hemorrhages in monkeys following exposure to acetonitrile vapor at 330, 660, and 2,510 ppm for 7 hours/day until death.

Subchronic studies involving acetonitrile have been reported for a variety of animal species. Pozzani et al [35] described the results of exposing monkeys to acetonitrile at 330 and 2,510 ppm for 7 hours/day. The monkey exposed at 330 ppm died after 2 days of exposure; the other monkey was alive after 99 days of exposure. At 330 ppm, monkeys showed subdural hemorrhage, brain congestion, and pleural adhesions. In the same study, rats were exposed to acetonitrile for 7 hours/day, 5 days/week at 166 and 665 ppm. No effects were observed at 166 ppm; however, rats exposed at the higher dose developed lung edema and congestion, bronchial inflammation with hypersecretion of mucus, and kidney and liver damage. Dogs exposed to acetonitrile at 350 ppm for 91 days showed a reduction in hemoglobin and hematocrit [35].

Acute and subchronic animal studies indicate that the effects of acetonitrile are species specific and depend on dose and duration of

exposure. Among the species studied, dogs were most resistant followed in decreasing order by rats, guinea pigs, and rabbits. Inhalation of acetonitrile produced the characteristic signs and symptoms of cyanide intoxication as described previously. The signs and symptoms reported are similar for long- and short-term studies. However, at relatively small but nonetheless lethal doses, death was delayed in all species. As with human case studies, these effects have been attributed largely to the nitrile per se and metabolic release of hydrogen cyanide [35,55].

Acute inhalation studies have been performed to determine the effects of cyanohydrins, notably acetone cyanohydrin and glycolonitrile. Wolfsie [40] reported that exposure to glycolonitrile at 27 ppm for 8 hours caused death in 29% of rats and 86% of mice studied; however, none of the guinea pigs in the study died from exposure. In another study [2], 50% of the rats died within 10 minutes following exposure to saturated vapor of acetone cyanohydrin.

Acute and subchronic inhalation studies have been conducted on selected dinitriles, notably tetramethylsuccinonitrile and malononitrile. In acute studies, Harger and Hulpieu [77] reported death of rats following exposure to tetramethylsuccinonitrile for 30 hours at 6 ppm. Reinl [43] reported that the mean death times for mice exposed at 0.158 mg/liter and 0.125 mg/liter were 153 minutes and 175 minutes, respectively. All animals died within 200 minutes.

In subchronic studies, Panov [68] reported that mice and rats exposed to malononitrile at 200-300 mg/cu m for 2 hours showed restlessness, an initial increase in the respiration rate, and lassitude, followed by decreased rate of respiration, incoordination, and convulsions. Death occurred in 50% of the rats studied. Results of another study [69] showed that, in rats exposed to malononitrile at 36 mg/cu m for 2 hours/day for 35 days, a decrease in the concentration in blood of hemoglobin and an increase in that of reticulocytes occurred.

Results of acute dermal studies [2,35,40] show that selected mononitriles, cyanohydrins, and dinitriles produce adverse effects in laboratory animals. Acetonitrile has been reported to be absorbed through intact skin of rabbits yielding a dermal LD_{50} of 980 mg/kg. Acute studies with glycolonitrile showed that rabbits exposed dermally at 105-130 mg/kg developed mild skin irritation. The LD_{50} for guinea pigs dermally exposed to acetone cyanohydrin was calculated to be 0.15 ml/kg.

Although exposure to nitriles in the workplace occurs primarily through inhalation and skin contact, additional effects have been observed in humans and animals with other routes of exposure, notably ocular, sc, iv, and ip.

Szabo (S Szabo, written communication, May 1978), in comparative toxicity studies with female rats, determined approximate LD_{50} values for

several nitriles. These values ranged from 100 mg/kg for malononitrile to 300 mg/kg for isobutyronitrile. Other nitriles tested were adiponitrile, n-butyronitrile, propionitrile, and succinonitrile. When compared with data on acetonitrile [2,35], Szabo's data provide a quantitative base for developing recommended concentration limits (Table III-4).

Panov [68] reported severe eye irritation and death in four of six rabbits following instillation of two to three drops of malononitrile into the conjunctival sac. In another study [40], three rabbits showed local eye irritation and convulsions within 15-30 minutes of applying glycolonitrile to the eyes. Death occurred within 68 minutes.

Animal studies indicate additional effects of nitrile poisoning, including the development of duodenal ulcers in rats administered propionitrile sc at doses from 15 to 60 mg/100 g for 4 days [56]. Nuclear changes in neurons and satellite spinal ganglia were seen in rats administered single doses of malononitrile (route unspecified) of from 6 to 8 mg/kg [70]. Thyroid hyperemia and hyperplasia have been reported in rats and rabbits administered acetonitrile sc at daily doses of 0.02-0.08 cc for 28 days and of 0.1-0.15 cc for 63 days, respectively [48,49].

Since human and animal studies mainly report signs and symptoms characteristic of cyanide poisoning, it is reasonable that a portion of the toxic effects of exposure to selected mono- and dinitriles is due to the release of the cyanide ion from the parent compound. The graded severity of effects observed in both human and animal studies indicates that the toxic action of nitriles depends on the dose and duration of exposure. The route of administration does not appear to be a major factor contributing to toxicity in that similar symptoms were seen in humans regardless of the route of administration [2,10,28,29,31-33,35-38,42].

Cyanohydrins and dinitriles show signs and symptoms resembling those reported for mononitriles. However, the dinitriles and cyanohydrins appear to be more toxic in that adverse effects generally occurred at lower concentrations. In addition, on acute exposures the cyanohydrins reportedly produced effects within minutes. Dinitriles could presumably exert a greater effect due to more rapid release of C≡N from one of the two nitrile groups available. As with selected mononitriles, reported effects of cyanohydrins and dinitriles may be due to the nitrile itself, to metabolites other than free cyanide, and to release of free cyanide.

Carcinogenicity, Mutagenicity, Teratogenicity, and Effects on Reproduction

No reports have been identified that discuss possible carcinogenic, mutagenic, or teratogenic effects of the selected nitriles, except adiponitrile. Adiponitrile has been tested in *Salmonella typhimurium* strains TA 1535, TA 1537, TA 1538, and TA 98 at concentrations up to 10,000

μg/petri plate [4]. No significant increase in the spontaneous (background) mutation rate was observed. Adiponitrile was not mutagenic in these microbial assays in either the presence or the absence of a liver microsomal enzyme preparation (5-g mixture). No adverse reproductive effects were found after one generation in rats exposed daily for two years to adiponitrile at concentrations up to 500 ppm [78].

A structurally related nitrile, acrylonitrile (vinyl cyanide) is suspected of inducing cancer in both animals and humans [89] (29 CFR 1910.1045). Since other vinyl derivatives containing electronegative groups, such as vinyl chloride and vinyl bromide, are also known to induce cancer, the adverse effects induced by acrylonitrile are probably more closely related to the vinyl moiety and not to the nitrile portion of the molecule. However, further scientific evaluation is necessary to confirm this conjecture.

TABLE III-2

EFFECTS OF SELECTED NITRILES ON HUMANS

Substance	Route and Duration of Exposure	Concentration or Dose	Number Exposed	Effects	Reference
Acetonitrile	inhalation, 4 hr	160 ppm (270 mg/cu m)	2	Bronchial tightness in 1	35
"	"	80 ppm (130 mg/cu m)	2	None	35
"	"	40 ppm (70 mg/cu m)	3	Slight bronchial tightness in 1	35
"	inhalation, unknown	Unknown	1	Gastric distress, respiratory distress, coma, death 6 d postexposure	33
"	inhalation, 2.5-5.0 hr	"	1	Hypotension, hypersecretion of saliva, conjunctivitis	34
"	"	"	15-20	9 became ill; 1 died; 1: chest pain, gastric distress, skin discoloration, tachypnea; 8: hypotension, general weakness, absence of deep reflexes, skin discoloration, tachypnea	31,32
Isobutyronitrile	inhalation, unknown	"	3	Dizziness, vomiting	10
Glycolonitrile	dermal,* unknown	"	2	General weakness; respiratory distress; unsteady gait; irregular, rapid pulse	40
Acetone cyanohydrin	"	"	4	1: nausea, convulsions, death 6.5 hr postexposure; 3: vomiting, cardiac palpitation	2
"	"	"	1	Vomiting, dyspnea, convulsions, coma	39
"	dermal,* 40-60 min wet and 5.5 hr dry	"	1	Constricting throat pain; slow, deep respiration; convulsions; cyanosis	42
"	oral, unknown	"	1	Death 12 hr postexposure	2
Adiponitrile	"	"	1	Vomiting, severe asthenia, tightening of the chest, tachypnea, raspy breathing, convulsions, cyanosis	16
"	dermal, unknown	"	7	1: skin inflammation, destruction, necrosis; 6: minor skin irritations	10
Malononitrile	iv, average of 48 min	2.4 mg/kg	40 (av 8 treatments each)	Tachycardia, congestive flow of blood to head	29
"	iv, 21-60 min	3-6 mg/kg	6	Tachycardia, vomiting, retching	28
"	iv	2-4 mg/kg	13 (9 had 10 or more treatments)	Tachycardia with palpitations, hypotension, gastric distress	27
"	"	1-6 mg/kg	66 (3-17 treatments)	Tachycardia, muscle spasms, gastric distress, convulsions in 2	26
Succinonitrile	im	200 mg	1 (19 daily) treatments)	Convulsions followed by death	30
Tetramethyl-succinonitrile	inhalation, unknown	Unknown	16	CNS and gastrointestinal disturbances, convulsions in 5, unconsciousness in 2	43

* With possible inhalation

TABLE III-3

EFFECTS OF SELECTED NITRILES ON ANIMALS

Substance	Route and Duration of Exposure	Concentration or Dose	Species	Number Exposed	Effect	Reference
Acetonitrile	inhalation, 30 min	53,000 ppm (89,000 mg/cu m)	Rats	-	Death in 50%	35
"	"	25,000 ppm (42,000 mg/cu m)	"	3	Dyspnea, cyanosis, 100% mortality in 30 min	55
"	inhalation, 4 hr	32,000 ppm (53,760 mg/cu m)	"	30	Death in 57%	79
"	"	8,000 ppm (13,440 mg/cu m)	"	30	Death in 33%	79
"	"	4,000 ppm (6,720 mg/cu m)	"	30	Death in 10%	79
"	"	16,000 ppm (27,000 mg/cu m)	Dogs	3	Death 14 hr postexposure	35
"	inhalation, 2 hr/d for 5 d	2,800 ppm (4,700 mg/cu m)	Rats	3	Dyspnea; anuria; hemorrhages in brain and lungs; death in all 3 rats	55
"	inhalation, 7 hr/d	2,510 ppm (4,200 mg/cu m)	Monkeys	3	Death after second exposure	35
"	"	660 ppm (1,100 mg/cu m)	"	3	Death after 23 and 51 exposures	35
"	inhalation, 7 hr/d 5 d/wk for 90 d	655 ppm (1,100 mg/cu m)	Rats	30	Bronchial inflammation, desquamation and hypersecretion of mucus, hepatic and renal lesions	35
"	inhalation, 7 hr/d 5 d/wk for 91 d	350 ppm (590 mg/cu m)	Monkeys	3	Bronchitis, post mortem: moderate hemorrhage of superior and inferior sagittal sinuses of the brain	35
"	"	"	Dogs	3	Decreased hematocrit and hemoglobin	35
"	inhalation, 7 hr/d	330 ppm (550 mg/cu m)	Monkeys	1	Excitability, post mortem: subdural hemorrhage, chronic pneumonitis, pleural adhesions	35
"	"	"	Rats	30	Bronchitis, pneumonia, atelectasis	35
"	"	166 ppm (280 mg/cu m)	"	30	Histiocyte clumps in alveoli of lungs	35
"	sc, 34 d	0.98-3.93 mg	Mice	12	Slight thyroid reaction	49
"	sc, 36 d	15.7-796 mg	Rats	12	Thyroid hyperemia and hypertrophy	49
"	sc, 20-63 d	79.6-118 mg	Rabbits	11	Thyroid hyperplasia, exophthalmos	48
Propionitrile	inhalation, 4 hr	500 ppm (1,125 mg/cu m)	Rats	6	Death in 33% after 14 days	80
"	sc, 4 d	15-60 mg/100 g/d	"	40	Duodenal ulcers	56
"	sc	6 mg/100 g	"	Unknown	Duodenal ulcers, gastric cellular changes	58
"	sc, 3 x d	60 mg/kg on d 1, 80 mg/kg on d 2, 100 mg/kg on d 3 and d 4	"	10	Duodenal ulcers in 80%; death in 100%	62
n-Butyronitrile	inhalation, 4 hr	1,000 ppm (2,830 mg/cu m)	"	6	Death in 83% after 14 days	81,82
"	"	500 ppm (1,415 mg/cu m)	"	6	No deaths after 14 days	81,82

TABLE III-3 (CONTINUED)

EFFECTS OF SELECTED NITRILES ON ANIMALS

Substance	Route and Duration of Exposure	Concentration or Dose	Species	Number Exposed	Effect	Reference
Isobutyronitrile	inhalation, 4 hr	1,000 ppm (2,830 mg/cu m)	"	6	Death in 100% after 14 days	81
"	"	500 ppm (1,415 mg/cu m)	"	6	No deaths after 14 days	81
Glycolonitrile	inhalation, 8 hr	27 ppm (63 mg/cu m)	Mice	7	Death in 86%	40
"	"	"	Rats	7	Death in 29%	40
"	"	"	Guinea pigs	7	No deaths	40
"	dermal, single	105-130 mg/kg	Rabbits	Unknown	Mild skin irritation, death in 50%	40
"	oral, 13 wk	62-92 mg/kg/d	Rats	"	None	40
"	ocular, single	55 mg of 50% solution	Rabbits	3	Moderate eye irritation, convulsions and coma 15-30 min postexposure, death in 100% 68 min postexposure	40
Glycolonitrile, 70%	inhalation, 4 hr	250 ppm (5,825 mg/cu m)	Rats	6	Death in 66% after 14 days	81
Acetone cyanohydrin	inhalation 3, 5, 8 mo	1 ml/84 liters	Rats	50	Bronchial ulceration, renal and hepatic necrosis	66
"	inhalation, 4 hr	125 ppm (435 mg/cu m)	"	6	Death in 100% after 14 days	81
"	"	62.5 ppm (217.5 mg/cu m)	"	6	Death in 33% after 14 days	81
"	oral, 3, 5, 8 mo	5 mg/rat	"	50	Gastric ulceration, liver necrosis	66
Malononitrile	inhalation, 2 hr/d for 1 mo	36 mg/cu m	"	10	Increased respiration, increase in reticulocytes, increase in weight coefficient of lung	69
"	dermal, single	Unknown	Mice	Unknown	Tachypnea, cyanosis	68
"	"	"	Rabbits	4	Trembling, erythema	68
"	ocular, single	5%	"	6	Severe eye irritation, death in 67%	68
Adiponitrile	sc, single	50 mg/kg	Guinea pigs	20	Tachypnea, irregular respiration, paresis, tonic contractions of extremities	16
Tetramethyl-succinonitrile	inhalation, 98-164 min	159 mg/cu m	Mice	5	Muscle spasms, death 98 min postexposure, death in 100% 164 min postexposure	43
"	inhalation, 130-200 min	125 mg/cu m	"	"	Muscle spasms, death 130 min postexposure, death in 100% 200 min postexposure	43
"	inhalation	60 ppm (334 mg/cu m)	Rats	Unknown	Death after 2-3 hr	77,83
"	"	6 ppm (33 mg/cu m)	"	"	Death after 30 hr	77,83
"	oral, unknown	49-56 mg/kg	"	2	Convulsions 5 hr postexposure, death several hr later	77

TABLE III-4

LD_{50} VALUES FOR MICE, RATS, GUINEA PIGS, AND RABBITS

Substance	Species	Route of Exposure	Concentration or Dose	Reference
Acetonitrile	Rats	inhalation, 4 hr	16,000 ppm (26,880 mg/cu m)	35
"	"	oral	2,460 mg/kg	79
"	Mice	ip	520.79 mg/kg	84
"	Guinea pigs	inhalation, 4 hr	5,655 ppm (9,500 mg/cu m)	35
"	Rabbits	"	2,828 ppm (4,751 mg/cu m)	35
"	"	dermal	1.25 ml/kg undiluted, (980 mg/kg), 75% solution 0.50 ml/kg (390 mg/kg)	35
"	Rats	oral	1.7-8.5 ml/kg (1,340-6,680 mg/kg)	35
"	Guinea pigs	"	0.177 ml/kg (140 mg/kg)	35
Propionitrile	Mice	ip	33.73 mg/kg	84
"	Rats	oral	39 mg/kg	80
"	"	sc	150 mg/kg	*
"	Rabbits	dermal	160 mg/kg	80
"	Rats	oral	80 mg/kg	*
n-Butyronitrile	Mice	ip	45.75 mg/kg	84
"	Rats	oral	135 mg/kg as 0.5% in corn oil	81,82
"	"	sc	200 mg/kg	*
"	Rabbits	dermal	400 mg/kg	81,82
Isobutyronitrile	Mice	ip	<50 l/kg (38.6 mg/kg)	64
"	Rats	"	0.25 ml/kg (190 mg/kg)	64
"	"	oral	100 mg/kg	81
"	"	sc	300 mg/kg	*
"	"	oral	200 mg/kg	*
"	Rabbits	dermal	240 mg/kg	81
Glycolonitrile	Mice	oral	10 mg/kg	40
"	Rats	"	16 mg/kg	81
"	Rabbits	dermal	105-130 mg/kg	40
"	"	"	5.0 mg/kg	81

TABLE III-4 (CONTINUED)

LD_{50} VALUES FOR MICE, RATS, GUINEA PIGS, AND RABBITS

Substance	Species	Route of Exposure	Concentration or Dose	Reference
Acetone cyanohydrin	Mice	unknown	2.9 mg/kg	65
"	"	ip	8.39 mg/kg	84
"	Rats	oral	17 mg/kg	81
"	"	unknown	13.3 mg/kg	65
"	Guinea pigs	"	9 mg/kg	65
"	Rabbits	"	13.5 mg/kg	65
"	"	dermal	16 mg/kg	81
"	Guinea pigs	dermal	0.15 ml/kg (140 mg/kg)	2
Isobutyronitrile	Rats	oral	100 mg/kg	81
"	Rabbits	dermal	310 mg/kg	81
Malononitrile	Mice	oral	18.6 mg/kg	68
"	"	ip	12.9 mg/kg	85
"	Rats	"	25 mg/kg	68
"	"	oral	61 mg/kg	119
"	"	"	100 mg/kg	*
Succinonitrile	Mice	iv, im	50 mg/kg	73
"	Rats	"	250 mg/kg	73
"	Guinea pigs	iv	50 and 60 mg/kg	73
"	Rabbits	"	23 mg/kg	73
"	Rats	sc	250 mg/kg	*
Adiponitrile	Guinea pigs	"	50 mg/kg	16
"	Rats	"	200 mg/kg	*
"	"	oral	300 mg/kg	120,*
"	Mice	ip	40 mg/kg	118
Tetramethyl-succinonitrile	Rats	oral	30 mg/kg	43
"	Guinea pigs	"	17.5-25 mg/kg	43
"	Rabbits	iv	20 mg/kg	77
"	Rats	ip	17.5 mg/kg	43
"	"	sc	30 mg/kg	77
"	Guinea pigs	"	23 mg/kg	77

*S Szabo (written communication, May 1978)